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CONTENTS.

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ON THE DIFFERENTIAL REACTION TO VITAL DYES EXHIBITED BY THE TWO GREAT GROUPS OF CONNECTIVE-TISSUE CELLS.

By

Herbert McLean Evans and Katharine J. Scott,

From the Anatomical Laboratory of the University of California.

With eleven plates.

ON THE DIFFERENTIAL REACTION TO VITAL DYES EXHIBITED BY THE TWO GREAT GROUPS OF CONNECTIVE-TISSUE CELLS.[1]

INTRODUCTION.

Within the last decade a prodigious mass of accumulated observations on the blood and connective-tissue cells has undergone systematic critical revision. Clear-cut anatomical data have been secured. If it must be confessed that we have not yet solved fundamental problems in the origin and cause of differentiation of these various elements, we are at least informed as to the number and kind of such elements. Histological, pathological, and clinical observations have gained assortment and appraisal. In the possession of the histological studies of Maximow, the investigations of modern cellular pathologists, among whom we may name Marchand and Aschoff, and the hematological critiques of Turk, Naegeli, Pappenheim, and Weidenreich, we are justified in feeling that all of the ordinary histological facts have been clearly established. Further inquiries can be undertaken and will have significance by virtue of this preliminary information.

Perhaps no inquiries have received attention commensurate with that accorded the interrelations of the cells of the blood and supporting tissues; for, though both these cell groups have been accurately explored, we know as yet but little of their affinities, the one to the other, or of their exchanges, if such occur. The doctrine of a strict separation of these two broad cell categories has been sharply attacked. The connective tissue is the ancestral and the ontogenetic home of the blood, says Weidenreich, nor does it ever wholly lose its considerable blood-cell content in normal life. It is perhaps because of these intimate relations with the blood-stream that, to most histologists, the connective tissues present a more complex cellular picture than they actually possess; but, besides the blood, the development of certain of its cells for the storage of fat, and, again, the presence of histiogenous mast-cells, have also added complexity to the subject.

In the descriptions furnished by every modern investigation, although the fact has not yet "settled out" into text literature, the connective tissue is an exceedingly simple and constant substance, for but two great groups of cellular inhabitants occur in it. Originality can hardly be claimed for this statement, yet it deserves reiteration. Attention to the demonstration of lymphocytic emigrations from the blood-stream into the connective tissue and to the ensuing "polyblastic" transformation of these cells, or, again, the belief that "rhagiocrine" characters are originally universal in connective-tissue cells and may at any time

[1] The present communication constitutes the fifth of a series devoted to the phenomena of vital staining with the acid dyes by Herbert M. Evans and Werner Schulemann. Most of our vital dyes were secured in the course of this previous joint work of Evans and Schulemann. The world conflict and its crucial alinements of loyalty have taken an inevitable toll in that plan. But neither this nor any other possible calamity could absolve a deeply felt obligation to acknowledge the credit due this previously loyal and generous collaborator, with whom contact has been impossible for a period of over six years.

become so, would not appear to justify a withdrawal of attention from the fact that the connective tissue is typically composed of but two cell strains—the phagocytic and the fiber-forming elements, that no other elements can be said to constitute its invariable make-up, and that these elements are at an early time sharply differentiated and continue to be so throughout life. A long line of investigations by modern histopathologists has served us here, but particular clarity has come from the histological researches carried out by A. A. Maximow.

Recent studies with entirely new methods, for example, the beautiful cytological analyses of tissue cultures by M. R. and W. H. Lewis, and similar *in vivo* studies on Amphibia by E. L. and E. R. Clark, all support this simple idea. The application of coloring-matters to the living animal—the so-called intravitam staining—has furnished us with a unique method for the recognition not merely of fine structural but also of physiological cell differences. Very striking results have been obtained with them, especially as regards the connective-tissue cells, in the case of animals which have been submitted to treatment with trypan blue and with isamine blue. These dyes affect in a sharply different manner the two great cell groups of the connective tissue and enable one to segregate them, with far greater precision than has hitherto been possible, into two definite cell classes. It is unnecessary for us to maintain, however, that this striking reaction can serve as an utterly reliable criterion of cell species and of cell relationships or transformations. It is necessary only that we recognize in it a selective histo-pathological reagent of great beauty which separates sharply the strains of connective-tissue cells existing at any one time into two functional types without appreciable intergrades. From the standpoint of the physiological significance of cell differentiation, this fact justifies all the scrutiny we can accord it, but especially as it involves the whole question of the fundamental nature of the reaction between living protoplasm and these dyes. We have attempted, consequently, in the present memoir, to describe with appropriate detail the biological peculiarities of the two connective-tissue cell types as far as they may be disclosed by their differential behavior towards such substances as are embraced in the numerous dyestuffs of the acid-azo class.

It is a safe prediction that he who has practised the study of living cells will not willingly revert to other methods, except for their admitted value as controls.[1] It is a curious commentary on the history of histological research that the microtome has greatly discouraged methods of direct observation of the living or surviving cell, methods which may now bring to their aid modern improvements in optical equipment and the discovery of specific coloring methods with vital dyes. He who has prosecuted with patience such studies upon the connective tissue has forced upon him the conviction of its unique effectiveness in differentiating cell types where the fixed picture leads to confusion.

The prolonged study undertaken by one of us in conjunction with W. Schulemann has had for its aim a comparative knowledge of the effects obtained with the

[1] Perhaps the extreme to which devotion to the section method may lead one may best be exemplified by our citation of Maximow (1916), who is willing to declare the accurate and painstaking observations of the Lewises as "of but little importance," because their transparent cultures were not embedded and sectioned.

great group of vital acid-azo dyes. Under the ægis of Ehrlich's notions, we saw unique advantages in the analyses of the relation of chemical constitution to biological effect in these numerous compounds, and, above all, an opportunity to track out in this way the underlying nature of the vital-staining reaction. 'We have announced in preliminary papers our conviction that the magical staining effect whereby trypan blue and its dye relatives mark out certain cells and tissues, leaving others unaffected, is only a phenomenon of elective storage. We have stated that the phenomenon has affinities with the well-known phenomenon of phagocytosis, not only by virtue of the fact that the cells which stain most intensely (macrophages) are all true phagocytes, but also because both with large particles and with dye molecules we have an ingestion and storage of foreign material on the part of the cell. Yet vital staining with the azo dyes differs from phagocytosis in the fact that in the former phenomena the physical dimensions of the particles ingested all fall in the ultra-microscopic realm and indeed—by virtue of their variable, but often marked, powers of diffusion—cross the imaginary boundary which Graham set up to separate crystalloids and colloids. This viewpoint is best expressed in the recognition that in vital staining with the trypan dyes a segregating and concentrating mechanism is possessed by the protoplasm of all vitally stained cells, as well as a mechanism by virtue of which coarse particles (phagocytosis) or excessively minute ones (dypsocytosis) may be taken in.'

'However perfect the ability of stains to diffuse into living protoplasm, there must also be a segregating and concentrating mechanism at work or their presence within the cell will not gain any emphatic exhibition as "vital" granules. Ample demonstration of this is shown, for instance, by the epithelial cells of the mammary gland, which may secrete into the milk of mother rats enough of certain dyes to deeply color the intestinal contents of the suckling young and yet no deposits occur in the gland-cells through which the dye molecules have with certainty passed.[1] Our conclusions, indeed, run sharply counter to those proclaimed by Fischel, Goldmann, and others, who would have preferred to see in all vital staining an elective tingeing of integral protoplasmic constituents or cell organs; but they rest on the confirmatory evidence of the most careful cytological studies on material in which many dye substances and the most various dosage have been employed. They have, furthermore, been accepted by those who have recently tested them either by the use of experiments or of an especially advantageous material, as attested by the conclusions of Kiyono in his second paper, Downey, McClure, and E. L. and E. R. Clark. This paper, concerned solely with the behavior of the connective-tissue cells, rests to a great degree on the experiments of the last few years prosecuted for the sake of the other and more general end, and will have to antedate the larger publication by stating its main conclusions, at least so far as they bear on the interpretations which we present. A personal and peculiar concern of Professor Mall, who from the beginning aided with more than sympathetic

[1] According to the studies of Sutter in this laboratory, this is the case, for example, with dianil blue 2 R (1 molecule ortho tolidine diazotized and combined with 1 molecule Neville-Winther acid and 1 molecule of chromotrope acid), but it does not hold for trypan blue, where mammery epithelial deposits are the rule.

interest the inception and prosecution of much of this work, renders it an appropriate, even if inadequate, tribute to his memory.

The reactions of the connective-tissue cells can be understood best by adopting as a standard their usual behavior in an animal which has received three or four moderate peritoneal doses (1 to 3 c. c. of a 0.5 per cent aqueous solution) of trypan blue at intervals of three or four days and then is anesthetized in order to study pieces of subcutaneous areolar tissue. There are places where small, very transparent sheets of this tissue, of remarkable beauty and uniform thickness, can always be obtained, *e. g.*, the investiture of the muscles of the thigh or of the spine. They should be stretched very rapidly by "raying" out their edges, which, from instant desiccation, adhere at once to a clean glass slide. A single drop of Locke's or other isotonic solution has been previously placed on a cover, which is now inverted on the stretched mount with all possible speed, is rimmed with lanoline or paraffine, and the preparation pushed under the microscope lens, advantageously, but not necessarily, upon a warm stage. The entire procedure can be executed by two persons in a half minute and thus all danger of desiccation or other change of the cells obviated.

Exploration of the whole fragment by means of the low-power (80 to 100 diameters) should precede study with the oil, but the latter must be inaugurated as soon as an impression of the low-power appearance can be registered and no inconsiderable numbers of both cell types rapidly passed under the immersion lens in review. In no other way can a judgment of the average cell-picture be obtained, and, consequently, in no other way can high-power camera drawings or paintings (which must be done from the living tissue) be prepared with the conviction that we have chosen a representative condition. In addition to the method of examination in an indifferent medium (isotonic salt) which we have just described, it is also of great importance that skin samples from the identical area be counterstained "supravitally" with a freshly made normal saline solution of janus green B (1 : 10,000) and a similar solution of neutral red. Janus-green preparations should in some cases stand exposed to the air for two minutes in order that the reducing powers of the protoplasm be counteracted by continual access to oxygen; they are then covered, drained off, and rimmed for speedy exploration with the oil.

In preparations which are made in any of the above ways, two sharply separated types of connective-tissue cells in all places make their appearance. The entire ensemble of morphological characteristics which distinguish these two types enables us to identify them with certainty as histiogenous phagocytes (clasmatocytes) and as fibroblastic cells. But our criteria for cell distinction, best expressed perhaps by Maximow in his article of 1906, are supported and accentuated by the vital stain as in no other possible way. The former cells, which unfortunately can be said to have received a new designation from almost every student of their anatomy and physiology, we propose to designate simply as macrophages—understanding, it is true, that under this term a rather heterogeneous group of elements is included; for besides the *connective-tissue macrophages* at least two other impor-

tant categories of these cells occur, viz, the *endothelial macrophages* of the liver, lymph-glands, bone-marrow, and spleen, and the *free macrophages* of the peritoneal and other serous fluids. In the areolar tissue by far the greater bulk of the vital stain has affected these connective-tissue macrophages. They are the cells par excellence which are electively displayed by this method. Almost every possible size of spherical dye-bodies or granules frequently occurs, varying from those on the limits of visibility up to structures as large or larger than the nucleus. The greatest disparity in sizes of these "seats" of the vital-dye effects occurs when the dye administration has been rapid and the dosage large (maximum acute effect); but a prolonged low dosage, though not the lowest, may render them strikingly uniform in dimensions. We shall designate these structures as the *segregation-apparatus* of these cells. They correspond to the vacuoles and "grains de ségrégation" of Renaut, the "granules" and "vacuoles" of Lewis and Lewis, and the "chondriosomenapparat" of Tschaschin. We believe we can show them to be a mechanism elaborated under functional stress for the temporary storage of these compounds.

The fibroblasts, larger, thin, flattened, almost perfectly transparent elements, possess in their cytoplasm, otherwise glassy-clear,[1] a segregation-apparatus so different from that of the macrophages that it has been misunderstood and wrongly identified with the mitochondria by Tschaschin.[2] Suffice it to say that the fibroblastic vital deposits of dye are always more minute and less perfectly spherical than those characterizing the macrophages, and that in the case of most dyes these minute deposits are often linear in morphology. (See figs. 73, 1, 2, 3, 4.)

Protocol: Rat 10, injected intraperitoneally with aqueous solutions of 0.5 per cent trypan blue, the combination, now well known, of o-tolidine with 2 molecules of the 1.8 amido-naphthol 3.6 disulphonic acid (H acid). March 1 to 7, inclusive, 2 c. c. each day.

March 7: Animal is deeply stained. Under the low-power, macrophages appear filled with bright-blue deposits, while fibroblasts can barely be seen as containing fine blue granules (fig. 1).

Under the oil, identification of cell types is confirmed. Macrophages have numerous bright-blue deposits which are practically all vacuolar. Crystals of dye occur rarely. Fibroblasts have fairly numerous, typical, minute deposits which tend to be linear, but are not the elongated thread and crystal forms found in chronic stains.

Protocol: Rat 31–2, injected intraperitoneally with fresh aqueous solutions of 1 per cent trypan blue, February 8, 11, 14, and 17, 1 c. c. each day.

February 19: Animal is stained a deep pure blue. Under the low-power, cell types can be distinguished.

Under the oil, macrophages are distinguished by their more abundant dye content and vacuolar structures, which vary greatly in size and in many of which crystals are condensing (fig. 3). Neutral red (1:5,000) gives a specific reaction with the vacuolar segregation-apparatus. It shows that the vacuoles have sickle-shaped accretions along one edge and that as a rule few or no crystals free in the protoplasm occur. Instances of their protrusion from vacuoles are, however, occasionally to be seen. Janus green (1:5,000), exposed 30 seconds and covered, showed undoubted mitochondria.

The fibroblasts in fresh preparations are characterized by minute deposits. Where the color is deep blue it is evident that these are crystals, but there are also many other minute, faint blue deposits which as a rule have an angular morphology. Neutral red brings out more clearly the fainter cytoplasmic deposits which before the counterstain was applied were not appreciated (fig. 4). No filar structures are encountered. Janus green (1:10,000), 3 minutes, showed a normal mitochondrial apparatus in the fibroblasts without staining the neutral red bodies.

[1] This is not quite true, since the filiform mitochondria of these expanded cells can always be distinctly seen by virtue of their difference in refractive index from the remaining protoplasm.

[2] Indeed, leading even to the derivation of the macrophage segregation-apparatus from mitochondrial

Protocol: Rat 333, injected intraperitoneally with fresh aqueous solutions of 0.5 per cent trypan blue, February 21 to 24, inclusive, 2 c. c. each day.

February 26: Animal stained a deep blue. Subcutaneous tissue deep blue. Under the low-power an elegant distinction of cell types is evident.

The oil shows this to be founded on the smaller, sparser, punctate, and linear nature of the fibroblast deposits in contrast to the macrophages, which are here characterized by fairly uniform-sized (somewhat larger than mast-cell granules) vacuoles which fill the protoplasm, though not so densely crowded as is often the case, and by a considerable number of somewhat smaller, refractive, fat droplets (fig. 2). Neutral red (1:1000) gives its specific reaction, and under the low-power marks the macrophages out with great emphasis owing to the accentuation of color. Janus green (1:10,000) did not stain the mitochondria of the macrophages; 1:5,000 (exposed 1 minute) finally showed a specific reaction in a few macrophages. Short, green, curved threads were seen lying between the vacuoles which were slightly pink from the janus.

The fibroblasts contain fairly numerous minute dye deposits. Some are granular, but the great majority are linear, or elongated triangles. Neutral red superimposes itself on these vital-dye deposits and, in particular, colors electively the linear endings of vacuoles, or the free linear structures, in the protoplasm. Janus green (1:10,000), exposed 10 minutes, stains typical mitochondria, but since the morphology, and especially the color, of the fainter, vital-dye deposits is so nearly identical with these, the distinction is well-nigh impossible, and one's belief in the success of the reaction is dependent upon the sudden and evident increase in the number of bodies having mitochondrial characteristics after the application of janus to the cell.

By the discovery of dyes which in fibroblasts, at variance with the description which we have just given, establish a typical vacuolar segregation-apparatus (figs. 44, 5) similar to that of macrophages, and, again, by the discovery of dyes and of dosages which greatly exaggerate the filiform modification (figs. 7, 9, 11) of the fibroblastic segregation-apparatus, we believe it has been possible to show points of fundamental similarity in the reaction of these two cell types, as well as those where characteristic differences are evident. Our line of argument will be facilitated by a preliminary analysis of the phenomena of "vital staining" as displayed by the macrophage cells.

It will be recalled that Ehrlich, to whom we are deeply indebted for the initiation of the whole field of inquiry into the selective effects of dyestuffs, saw in vital staining with the acid dyes an ocular demonstration of his chemoceptor theory in the domain of the new science of chemical therapeutics.[1] It will suffice as an answer to this conception for us to state that the chemoceptor theory would appear to have been adequately disproved by the above-mentioned experiments which one of us has carried out over a considerable period of time in conjunction with W. Schulemann and in which several hundred compounds, whose approximate chemical constitution was known, were tested for their biological effect. Some 260 of these dyestuffs were known to us, as far as their chemical structure was concerned, with all possible accuracy. Our studies showed the inadequacy of any such theory in enabling us to predict either the spread of the dyes throughout the body (the macroscopic vital stain) or the exact type of reaction which would be produced with the vitally stained cells. Indeed, our investigations indicated that various physical characters of the dye solutions were the determining factors in their so-called "staining" effects.

[1] Ehrlich, Paul, 1913. Address in Pathology on Chemiotherapy, delivered before the Seventeenth International Congress of Medicine, British Medical Journal, 1913, vol. 2, pp. 353–359: "I will mention here—in order only to indicate a few examples—the intra-vitam staining of the nerve trunks by methylene blue, the staining of cell granules by neutral red, and the distribution of isamine blue in the so-called pyroll blue cells so carefully and excellently investigated by Edwin Goldmann."

When now we enter the realm embraced by studies on the physical peculiarities of dyes in relation to their power to affect the living cell, we are confronted at once by the predominant theory in this field, that of Overton, which would recognize solubility in lipoids as a necessary possession of any coloring substance which can gain entrance into the cell. If adequate as an explanation for the behavior of most of the vital stains originally discovered (methylene blue, neutral red), this theory must be abandoned in the case of staining with the great acid-azo group—dyes insoluble in lipoids, but soluble in water. There may, indeed, still be some pertinency in Overton's suggestion, inasmuch as the most rapid penetration by the basic dyes of the protoplasm of all cells may be referable to the solubility of these dyes in certain substances; certain it is that the acid-azo compounds gain admittance only to a precise set of cells and are apparently forbidden entrance into the great group of nervous, muscular, and epithelial tissues. Nor is the rate of entry of the acid dyes into the cells which they peculiarly affect (the macrophages) anything like as speedy a process as that shown by basic dyestuffs. In a few seconds after treatment with weak yellowish solutions of neutral red these cells concentrate in their segregation-apparatus deep red deposits of this dye, but they are unable to effect such a concentration of the acid dyes, even when swimming in high concentrations of the latter, except after an interval of several hours.[1]

It is apparent that, if the acid dyes enter the macrophages by free diffusion, the latter process is at least seriously impeded when compared with the diffusion of basic dyes. We have long been acquainted with a slower, though effective, manner of entry of substances into the living cell—phagocytosis—and it could seem no mere accident that acid-dye granules were exhibited predominantly by cells whose phagocytic properties have long been known (Metchnikoff, Renaut, Dubreuil). We have, indeed, through experiments with particles visible with the oil lens, and which, of course, settle quickly out of suspension (lampblack), with particles barely on the limits of visibility and which settle more slowly (India ink), with suspensions which are permanent and the particles of which are invisible except to the ultra-microscope (the metallic sols), and with high colloidal dyes of the acid-azo series, been in the possession of a series of substances whose physical dimensions start from those comparable with bacteria and which possess no power of independent movement, and, indeed, merely oscillate with Brownian motion, to particles whose existence can be displayed only by their opacity and the associated Tyndall phenomenon of their "solutions" or by the ultra-microscope, to end with particles which can penetrate membranes by dialysis and reach considerable distances through their power of free diffusion. All of these substances enter and are concentrated in the segregation-apparatus of the macrophage cells, where the conditions for their anchorage are sufficient to allow them to remain for a considerable time. While

[1] Winternitz and Evans have shown that as a consequence of mechanical injury (tapping of the cover glass) blood-cells are instantly, though diffusely, and palely stained by the acid dyes. It is probable that this change in behavior is due to the "setting" phenomena which occur on protoplasmic death and which immediately allow the free spread of acid dyes, though no concentration of the same. The latter is a phenomenon exhibited only by the living cell and is the only outstanding justification of the term "vital staining" when applied to dyes considered here, for their concentration in the protoplasm can only be carried out by the living cell. Loele (1912) has shown a similar great delay in the rate at which paramecia house pyroll blue in their vacuoles when compared with methylene blue.

the larger of these particles can be seen to enter the cell (after adherence to it) by the well-known engulfment act (phagocytosis), the smallest of them probably do so by virtue of their considerable, even though hindered, powers of diffusion, their penetration being much facilitated by the concentrating mechanism of the segregation-apparatus. This is unquestionably the mode of entry of the basic dyes, and the most diffusible of the acid dyes probably fail in the extent to which they give a "vital-staining reaction" when compared with the more colloidal members of the series, not through their lack of power to enter the cell, but through the equal facility with which they leave the same without gaining a segregation or fixation within the cell.

Inasmuch as it has been necessary for us to discuss the vital-dye deposits as in the "segregation-apparatus," it is appropriate to indicate briefly, now, the lines of evidence which have led us to this view and to discuss other conceptions of the nature of the vital-dye bodies. Both Fischel and Goldmann, who have furnished important explorations in this field, and Arnold, whose meticulous records occupy the endeavor of a lifetime, have championed the view that with vital dyes, cell organs, pre-existing but less clearly seen, are electively exposed to view. We believe we can nullify the "preformed-structure" argument speedily as far as it relates to the dye granules produced by acid-azo dyes. Modern cytological researches, both with fixed and with living material, have acquainted us too accurately with the cytoplasmic structures or cell organs of the connective-tissue cells for us to have overlooked the pronounced structures represented by the dye granules were they present in normal cells. We are acquainted with the centriolar apparatus, the mitochondria, and the "vacuoles de ségrégation" of Renaut (the normal segregation-apparatus). In the macrophages the dye deposits need never be confused with the first two of these cell organs and usually greatly exceed the normal limits set for the third.

In conformity with Renaut and Dubreuil (see also M. R. and W. H. Lewis), we have found that the normal segregation-apparatus of macrophages is electively displayed by neutral-red solutions (1: 10,000). The dimensions and extent of this system are subject to considerable variation, but under no circumstances does it approach the extent attained under acute dosage with any of the benzidine dyes (fig. 12). Most of the dye structures, consequently, must be interpreted as new formations, a fact which receives all the convincing proof one can desire in cases where an identical cell can be watched in its relation to the dye, *e. g.*, the migratory macrophage cells at the periphery of transparent tissue cultures or in the tail of amphibian larvæ, where many of the dye deposits can be seen in the act of origin *de novo*.[1] The "preformed-structure" conception, indeed, could hardly give us serious concern were it not for the astonishing paper written by Tschaschin, of Maximow's laboratory, the keynote of which is his attempted recognition of all vital-dye structures as mitochondrial derivatives or actual mitochondria, a view

[1] An even better instance is furnished by Clark's description of the large juxtanuclear accumulation of dye globules in the lymphatic endothelial cells of the tail of frog larvæ where previously these certainly did not exist.

which, surprisingly, has not been received with disfavor by some able investigators (Kiyono, Maximow). Tschaschin's line of proof for such a claim is singularly uncritical and consists essentially in a mere analogy between the picture furnished by methods for demonstrating the mitochondria in fixed preparations (unfortunately not highly specific) and the picture furnished by the minute, frequently linear, fibroblastic deposits. If this fact obtains for the fibroblasts (*i. e.*, the deposition of the dye on the mitochondria), reasons Tschaschin, then it also obtains for the macrophages, and the dye bodies here are merely transformed mitochondria.

We will take up the confusion which has obtained in the case of the fibroblasts at a later point in this paper. As regards the macrophages, aside from the fact that these cells normally possess a segregation-apparatus which, though small when compared with the "dye bodies," is at least nearer them in morphological characteristics than the mitochondrial system, there is also the clearly demonstrated fact, which Tschaschin overlooked, of the presence of a normal mitochondrial content in vitally stained macrophages. There is, indeed, no method which can equal in exquisite electiveness for the demonstration of mitochondria the supravital application of janus green B in strengths which vary from 1:100,000 to 1:10,000, but do not exceed the latter. Neither the iron hematoxylin, the fuchsin, nor the alizarine methods have in our hands furnished as satisfactory data as have studies on fresh tissue. Worked out in this way, the mitochondria[1] of the connective-tissue macrophages are seen to consist invariably of a series of granules, rods, and threadlets scattered throughout the protoplasm between the small vacuoles of the normal vacuolar-apparatus, tending perhaps to be somewhat more frequent in the vicinity of the nuclear membrane (fig. 14). No significant departure from this picture occurs in the case of fully laden vitally stained cells (figs. 15, 17, 19). The mitochondria have not disappeared in these cells, though they are often forced to occupy squeezed-in positions between large vacuoles which are almost contiguous and badly crowd the cytoplasm. These facts, stated by one of us in a previous preliminary communication, are, however, not always easy to demonstrate, but we believe we have disclosed the reason for this in the fact that if counterstaining with janus green be attempted while the tissue fluids are still full of the azo dye, a chemical precipitation between the acid and basic dyestuffs prevents the janus dye from exercising its specific effect unless it be employed in fairly high concentrations (1:5,000) and be several times renewed. In spite of the work of Dubreuil, Guilliermond, Azzi, and others on the share which the mitochondria take in the mobilization of natural pigments, glycogen, and fat (conceptions which the Lewises were unable to confirm), we must also range ourselves with the latter observers in so far as the deposition of acid dyes within these cells is concerned, for we have at no time found justification for Tschaschin's description of a modification of the mitochondria by virtue of their assumption of a dye-storage function.

The conception that the "dye bodies" are actual accumulations of vital dye in a partly hypertrophied, partly newly formed segregation-apparatus devised by the cell

[1] We see no reason to enrich the terminology by the employment, as some do, of special designations for these structures in accordance merely with their granular, bacillus-like, or thread-like form.

to receive them, rests essentially on four lines of evidence, which may be enumerated as follows:

1. The number and size of the dye granules, or stained structures, within the cell, other things being equal, increases with increased dye dosage.

2. Instances of metachromasia displayed by some few of these dyes prove conclusively that the dye slowly concentrates in the segregation-apparatus until it is thrown out of solution, the solid form showing a different color from the fluid.

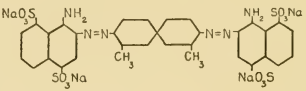
3. By appropriate dosage, a true crystallization of some of these dyes can be made to occur within the cell.

4. Double staining, *i. e.*, the superposition of one acid dye of marked color difference from a preceding one (the effects of which have been previously determined), demonstrates that both substances are handled together in the same place of deposit (*i. e.*, in the segregation-apparatus), unless the diffusion rate of the two dyes, ease of segregation or crystallization, or other physical attributes dispose them to produce a different vital-dye picture in the cell.

We may now consider in appropriate detail the nature of this evidence.

1. As regards the macrophages, very different effects are obtained in accordance with the method of administration of the same dye, and again, equally pronounced differences, in accordance with the dye chosen for experiment. In the case of many of those benzidine dyes which diffuse easily *in vivo*, a daily intraperitoneal administration of 1 c. c. of a 0.1 per cent solution leads only to the most gradual attainment of macroscopic color; nor are the macrophages in the areolar tissues of such an animal at all similar to those of an animal which has tolerated an acute high dosage of the same dye. It will be remembered that the acute high-dosage effect is to give us macrophages with large and small vacuoles which show great disparity in size (fig. 20). The macrophages of animals treated with very low dosages of the same dye are remarkable for the fact that their vacuoles are small and of strikingly uniform size (fig. 22).

Protocol: Rat 30, injected intraperitoneally with a 1 per cent solution of dye T 148, a brilliant red, diffusible, true vital stain, synthesized for our comparative studies on the azo dyes. The dye results from the combination in alkaline solution of 1 molecule of o-tolidine with 2 molecules of alpha naphthylamine 4.8 disulphonic acid, and hence has the formula



March 1 to 7, inclusive, 2 c. c. each day with the exception of March 5.

March 8: Animal is stained a deep red. General condition of animal somewhat affected by dye dosage. Subcutaneous tissues are stained light red. Under the low-power, more color is present in cells than is usual with this dye. The bright-red deposits seen in great numbers are apparently in macrophages, but fibroblasts are easily identified and contain a large amount of dye. They are not, however, as bright a red as the macrophages.

Under the oil, differentiation of cell types is confirmed. The reaction is essentially a macrophage reaction, *i. e.*, the macrophages contain the greatest amount of dye, as is shown in every way by the size and number of their vacuoles and by the deeper red color they possess. These cells vary

greatly in their size as well as in their dye-content. The larger macrophages, which are numerous, contain medium-sized, very regular dye vacuoles. The characteristic cell, however, is one often of great dimensions filled with red vacuoles which vary in size from minute structures to some even larger than the nucleus (fig. 20). Fat globules are numerous in these cells. Neutral red stains the dye vacuoles, forming crescentic concretions in those of medium size. Larger granular concretions are present in the large vacuoles. Janus green positive for mitochondria.

Fibroblasts are well filled with vacuoles of uniform size resembling mast-cell granules and almost entirely round. These are not red, as are the macrophage vacuoles, but have a decided pink color (fig. 21). Linear deposits are so rare as to be discounted. Neutral red stains the dye vacuoles and shows concretions in very many. No linear structures are seen with it. Janus green positive for mitochondria.

Protocol: Rat 18–2, injected intraperitoneally with a 0.5 per cent solution of dye T 148. January 31 to February 17, inclusive, 1 c. c. each day.

March 18: Films from thigh examined. Animal is stained deep rose-color. Subcutaneous tissues deep red. Under the low-power, all cells appear packed with pink deposits, among which one can see bright-red concretions even with this power. Macrophages can not be identified.

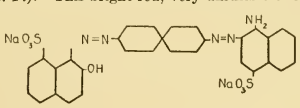
Under the oil, macrophages are at first exceedingly difficult to identify, but differ from the fibroblasts in having more round and slightly smaller dye vacuoles, among which crystals, though sometimes present, are never very numerous and are often lacking. If crystals occur in these cells, they are short; and often granular concretions are the only solid form found. The vacuoles are pale pink and colorless (fig. 22). Neutral red affords a method of identifying with certainty the macrophages, whose abundant vacuoles are stained specifically a bright orange, much deeper, than the staining present in fibroblasts. Janus green shows normal mitochondria.

Fibroblasts have an enormous number of vacuoles which are fairly uniform in size, are angular, and straw-colored. Among these and occasionally connected with them are straight, deep crimson-black crystals (fig. 23). These are sometimes curved about a vacuole. Crystals are very abundant, the typical cell containing two or three times as many as in the case figured. They are short, straight, almost black rods. There are no other linear deposits. Neutral red stains the vacuoles; janus green shows normal mitochondria.

March 28: Films of subcutaneous tissue from the upper abdomen show fibroblasts still engorged with small, pale-yellow T 148 vacuoles. While some have no other deposits in them, it is not difficult to find cells with the characteristic dark-red, long, bacillus-like crystals. The majority of the cells display a great number of these. The rods are unconnected with the vacuoles. They vary in length from 11 mm. to 2.5 mm., the average being perhaps 4 mm. or 4.5 mm. Instances of larger crystals in macrophages along the course of blood-vessels are seen, but it is uncommon to find appreciable dye in these cells.

Again, among the most soluble dyestuffs of this class may be encountered some whose speed of egress as well as ingress is so great that, even with relatively high dosage, large vacuoles are never created in macrophages, but only small ones, in a way similar to that we have just described as following the most dilute possible dosage with a typical positive dye. (See figs. 24, 25, 26, 27, 28, 29, 30, 31).

Protocol: Rat 34, injected intraperitoneally with 1 per cent aqueous solution of Baumwollrubin (B. A. S. F.). This bright-red, very diffusible compound has the formula



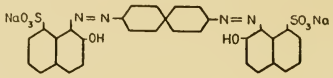
April 17, 18, 19, 20, 24, 25, and 26, 4.5 c. c. each day.

April 30: Animal is stained a rose-color. Skin of thigh is pink. Under the low-power, macrophages are well filled with pink deposits, while fibroblasts can barely be seen.

The oil shows macrophages filled with uniform pink vacuoles which are remarkably small. No concretions are present (fig. 24). Janus green is positive, giving a brilliant contrast to the dye vacuoles and remaining positive after the nucleus is stained a light purple, while the vacuoles are orange.

Fibroblasts contain a scantier number of vacuoles which are, on the whole, similar in size to those in macrophages, are often grouped at one or both poles of the nucleus, show considerable variation in size, and are almost invariably with some linear forms (fig. 25). There is a definite pink color in all the threads. With neutral red, vacuoles often appear strung along threads. Janus green is positive. The threads remain pink.

Protocol: Rat 47, injected intraperitoneally with 2 per cent aqueous solution of New Bordeaux L (B. A. S. F.). This, which is again a very diffusible compound, has the formula



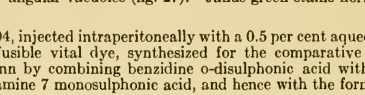
March 8, March 10 to April 5, inclusive, and April 7, 1 c. c. each day.

April 9: Animal is stained a fairly deep red. Subcutaneous tissue is light red. Under the low-power there is seen to be considerable dye in both types of cell. Macrophages are filled with red deposits and are easily distinguished from the paler fibroblasts.

Under the oil, macrophages contain an abundance of vacuoles which are about the size of mast-cell granules, but are not at all uniform in size, although the variations are slight. Some are more deeply stained than others. Neutral red stains these vacuoles. No definite concretions are present (fig. 26). Janus green stains normal mitochondria.

Fibroblasts are closely packed with vacuoles of uniform size. These are about the same size as those most frequent in macrophages and very close to that of mast-cell granules. They are a pale pink in color. In many cells "tails" are present on some of the deposits and a few cells contain numbers of typical thread-forms, but the great majority of the fibroblasts have deposits which are simple round or slightly angular vacuoles (fig. 27). Janus green stains normal mitochondria.

Protocol: Rat 94, injected intraperitoneally with a 0.5 per cent aqueous solution of dye 230, a red diffusible vital dye, synthesized for the comparative studies of Evans and Schulemann by combining benzidine o-disulphonic acid with 2 molecules of beta naphthylamine 7 monosulphonic acid, and hence with the formula



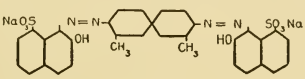
April 11 to 20, inclusive, 1 c. c. each day.

April 23: Animal stained a dusky rose. Skin of thigh pink. Under the low-power no difficulty is experienced in distinguishing the cell types on account of the almost complete lack of visibility of the fibroblasts. The deposits in the macrophages are easily seen. They are relatively small for this cell type and intense red.

Under the oil, the macrophages are characterized by the possession of small vacuoles in which the tendency to deep-red concretions which are punctate or sickle-shaped is almost universal (fig. 28). There are many small deep-red punctate deposits which can be demonstrated to reside in larger vacuoles. It is also true that neutral red fails to show other vacuole deposits about many of the sickles. Neutral red shows some vacuoles about the diameter one would expect from the sickles without a visible amount of dye 230, but these are infrequent. It is evident that this dye resembles vital new red in producing only small vacuoles in the macrophages in which the tendency to condensation is great, but the form which these condensations take differs from that of vital new red. They are here small, circular concrements and rarely short rods. Janus green 1:10,000 (5 minutes) gave a brilliant stain of normal mitochondria.

The fibroblasts are characterized by the extremely pale dye-content of their small, angular vacuoles, except where punctate, deep-red concretions occur. Linear structures, though not occurring in all cells, are frequent. These vary from comet elongations of the vacuoles to long, truly filamentous structures in the course of which widenings or deep-red concretions may occur. Occasionally the concretions in the small vacuoles may take the form of short red rods. The deep-red concretions in relation to "threads" are sometimes dispersed along the thread (fig. 29). The dye deposits in the fibroblasts are on the average similar in morphology to those characterizing vital new red, but they are more abundant and they are very much paler in color (so much so that they are overlooked at low power). Janus green stains filiform and granular mitochondria unconnected with the dye deposits.

Protocol: Rat 19, injected intraperitoneally with a 1 per cent solution of dye 1212 *d*, a very diffusible bright-red dye synthesized for us by combining 1 molecule of o-tolidine with 2 molecules of beta naphthol 8 monosulphonic acid, and hence with the formula



May 3, 6 to 14 inclusive, 16, 18, 20, and 22, 1 c. c. each day.

May 24: Animal is stained a light pink generally. The skin of the thigh is light pink. Low-power shows macrophages as faint-pink cells. The fibroblasts are not appreciably stained.

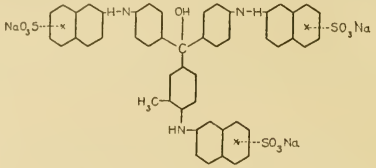
The oil shows that the macrophages, though not enlarged, are full of small, uniform pink vacuoles without condensations or much variation in size, though the form is irregular (fig. 30). Neutral red gives its customary reaction with the macrophages. Janus green gives a specific stain of mitochondria.

The fibroblasts have a very much scantier content of dye, which consists of fair-sized, angular deposits dispersed in a more scattered fashion and extremely pale pink (fig. 31). Their size compares well with that characterizing the macrophages. Janus green shows normal mitochondria in brilliant contrast to pink dye deposits.

The abdominal skin is deep red, the macrophages containing very great numbers of small vacuoles which are uniform pink and the fibroblasts containing several times the content of those in the thigh, so that these cells are at length appreciated with the low-power as little trails of granules, but the fibroblastic cytoplasm is still far from filled.

When, now, we turn to the behavior of negative dyes, *i. e.*, those which have greatly restricted powers of diffusion and remain chiefly in the immediate area where they are injected, the size and number differences in the macrophage deposits vary in strict accordance with the proximity or distance of the cells in question from the injection-point. (See figs. 32, 33, 34.) The macrophages of the subcutaneous tissue of the head, in animals receiving repeated middorsal subcutaneous dosage with isamine blue (a sulphonated triphenylmethane dye), contain only fair-sized homogeneous globules of the stain, but those near the point of application of the dye and in the area of its immediate spread, after massage, are often gigantic in size. The free peritoneal macrophages of animals which have received intravenous injections of any of these dyes never contain as large or as abundant "dye bodies" as they do on direct injection of the dye into this cavity. We shall refer subsequently to the fact that quantitative changes to an even more pronounced degree occur in the segregation-apparatus of the fibroblasts under similar conditions, and we have indicated what we feel is the significant import of this evidence for our theory of the similarity in nature of the dye structures in both fibroblasts and macrophage cells.

Protocol: Mouse 36, October 26, injected subcutaneously with 0.5 c. c. of a 1 per cent solution of isamine blue 6B, a sulphonated triphenylmethane dye, with the formula



x—exact position of sulphonic radical uncertain.

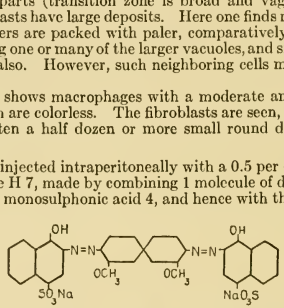
November 3: Ears are pale blue; skin over back and sides is deep blue; subcutaneous tissue definite pale blue on side opposite injection-point.

Injection-site contains many macrophages with typical form and deposits of varying sizes. Fibroblasts are in the majority well filled with large dye vacuoles, though these same cells may show processes quite free from dye as well as many small vacuoles in parts.

In less heavily stained parts (transition zone is broad and vague), the cell types are more distinct, though many fibroblasts have large deposits. Here one finds rounded-up macrophages with immense vacuoles, while others are packed with paler, comparatively small deposits. The latter, however, are found containing one or many of the larger vacuoles, and similarly those with the largest vacuoles have some smaller also. However, such neighboring cells may be strikingly dissimilar in general appearance.

The palely stained area shows macrophages with a moderate amount of dye, in the form of small vacuoles, some of which are colorless. The fibroblasts are seen, even under the oil, with more difficulty. They contain often a half dozen or more small round dye granules unrelated to the mitochondrial threads.

Protocol: Rat 25–1, injected intraperitoneally with a 0.5 per cent solution of the deep-blue "negative" dye H 7, made by combining 1 molecule of dianisidine with 2 molecules of alpha naphthol monosulphonic acid 4, and hence with the formula



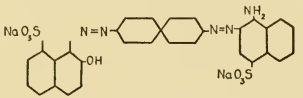
February 1, 4, 8, 11, 14, 17, 20, 23, 26, March 1, 4, 7, 10, and 13, 2 c. c. each day; March 17, 19, 22, and 25, 4 c. c. each day.

March 27: Skin of scrotum is deep blue; lower abdominal skin has a pale-violet hue. The animal is otherwise unstained. Under the low-power the skin of the thigh shows macrophages with a very pale blue content of dye. It is only under the oil that one finds the two or three pale-blue deposits that are present in most fibroblasts. Even with neutral red the macrophage reaction is predominent and fibroblasts can not be detected with the low-power and with the oil their vacuoles are lacking in intensity and are scanty in number, but it is certain that the number of the neutral-red bodies is somewhat above normal (fig. 33). Janus green shows normal mitochondria.

Macrophages of the thigh have a dye-content of a fair number of vacuoles, small and round, of uniform size, in which neutral red shows frequent concretions (fig. 32). In the scrotum these cells are filled with vacuoles of an intense blue color, small, however. At the puncture-point mammoth cells are present, with large vacuoles of deep purple among smaller, more uniform sized vacuoles which are still larger than those found in the thigh (fig. 34).

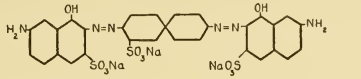
In the skin of the scrotum, fibroblasts are distinguished by the enormous number of small round and angular dye bodies which fill the cytoplasm and by the concurrence of cells in which many blue linear forms are present. These threads carry dye concretions at their ends and along their course. Cells of this type occur also at the puncture-point, but are not found more than a few millimeters from it. It is remarkable that they do not occur in the serous membranes, where an enormous number of small deposits crowds the cytoplasm.

2. The facts which one of us in conjunction with Schulemann was able to establish concerning the metachromasia exhibited by some of these dyes (Evans and Schulemann, 1915) have been examined by Schulemann (1915) with reference to the probability with which we can affirm chemical or physical causes to underlie this color change. It will suffice to remark here that what we have instanced as occurring with Congo rubine,



occurs with no less than 12 dyestuffs of the benzidine group,[1] in all of which there takes place to a variable degree a color change in the dyestuff stored in the segregation-apparatus in accordance with coaguluative changes in the high colloidal solutions there present.

Protocol: Rat 89, injected intraperitoneally with a 0.5 per cent solution of the lilac-colored dye 150 synthesized for the comparative studies of Evans and Schulemann by combining 1 molecule of benzidine monosulphonic acid with 2 molecules of 2-amido-8-naphthol-6-monosulphonic acid, and hence with the formula



April 11 to 26, inclusive, 1 c. c. each day.

April 28: Animal is stained a fairly deep lilac. Subcutaneous tissue is pale lilac. Urine is pale lilac. Low-power shows small bluish deposits in macrophages.

Under the oil, fibroblasts are not at first apprehended, but on careful searching it is possible to make sure that these cells carry a scanty content of small, pale-lilac vacuoles with some denser *deep blue.* Neutral red shows an increased number of small vacuoles, but never gives any large number, even in the most crowded cells (40 to 80). Janus green shows normal mitochondria.

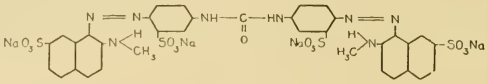
The macrophages are distinghished by the possession of small blue-lilac deposits, which are in the form often of *deep-blue concrements* in vacuoles. Some of the larger vacuoles show a distinct reddish tint. Neutral red discloses a fair number of small vacuoles not seen before. Janus green shows normal mitochondria.

These coagulative changes, concretions, or concrements, as we have variously designated them, are again to be separated from true crystallization processes which may or may not be associated with change of color, and to which we will now refer.

3. In the employment of dyestuffs which have such a colloidal state that even minute quantities of them, after their segregation from the protoplasm, remain anchored in the cell with great permanency, we have the means at hand to produce, by very dilute dosage, minute dye deposits which are almost entirely true concrements, while no significant enlargement of the segregation-apparatus occurs. (See figs. 28, 35, 36, 37.)

[1] The behavior of these substances is identical in principle with that shown by Congo rubine. They were to be described in full in our larger publication ("The Phenomenon of Vital Staining with Acid Azo Dyes," Evans, Schulemann, and Wilborn) and are given by Schulemann (1917). It is remarkable that von Möllendorf (1914) has sought to interpret our proof that the dyes gradually solidify in their places of deposit as a supposition on our part that the dye deposits are always granules of the solid dye substance! We had described in explicit terms our conception of the formation of vacuoles in the genesis of vital granules in the protoplasm of vitally stained cells (Evans and Schulemann, 1915).

Protocol: Rat 10, injected intraperitoneally with a 0.5 per cent solution of vital new red, a brilliant orange-red tetrasulphonated azo dye with the following formula:



April 10 to 19, inclusive, 1 c. c. each day.

April 22: Animal is stained a brilliant crimson. Skin of thigh is a pale crimson. With the low-power, one is easily able to distinguish cell types, the trails of minute red granules representing fibroblasts being in conspicuous contrast to the agminations of larger, brilliant red granules which mark the macrophages.

Under the oil this distinction of cell types is confirmed. There are a relatively small number of deep-red angular bodies in the fibroblasts. The majority of these are small, but a few attain greater size and tend to resemble crystalline plates, though none is very large (fig. 37). Neutral red stains but few additional bodies, if any. Janus green stains normal mitochondria, affording a brilliant contrast to the red deposits.

The macrophages contain no very large deposits. The larger vacuoles, which show conspicuous peripheral agminations, are of fairly uniform size (fig. 35). Some are a diffuse bright red, but most of them show 3 to 7 or 8 bright-red, clearly outlined concrements of some refractivity. There are many concrements of this sort distributed free throughout the cytoplasm, having evidently arisen from minute vacuoles. Very few pale deposits can be found, most present being brilliant red. Neutral red shows a number of palely stained vacuoles which contain some of the apparently free concrements (fig. 36).

Protocol: Rat 101, injected intraperitoneally with a 0.1 per cent solution of vital new red. April 24, 27, 30, May 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, June 2 and 5, 1 c. c. each day.

July 8: Animal is stained a light pink. Subcutaneous tissue of thigh pink. Under the low-power, one finds minute, deep-red deposits apparently in all cells, but types can not be distinguished.

The oil confirms the impression that both cell types are involved; the granules are intense deep red, but there are always somewhat more in the macrophages than in the fibroblasts. Even in the macrophages these deposits have a somewhat angular morphology and are to be interpreted as concretions of dye. Neutral red 1:10,000 gives its customary reaction with the macrophages, making cell types much more easily distinguishable.

The fibroblasts contain from 8 to 25 of the deep-red dye concretions (the macrophages at least five times this number); but neither size nor morphology of the deposits varies with the cell types. Neutral red enables one, in the case of the fibroblasts, to ascertain that besides the dozen or more evident deep-red deposits there is an additional equal number of small vacuoles which now stain a weak orange. Janus green gives a brilliantly elective stain of mitochondria.

When, in addition to these adhesive traits, the dye substance is also one that easily crystallizes, the method of prolonged, very dilute dosage yields another set of unique effects, namely, the *appearance of minute, true crystals of the dye* within the cell. These have affected the pre-existing vacuolar apparatus of the macrophages but slightly, but the crystals are easily seen rimming part of the contour of a tiny vacuole as an intensely colored crescent. Many of them, however, condense at points evidently not previously the site of a vacuole, and consequently are built up free in the protoplasm, from which they are never separated by vacuolar walls. Such are the appearances when one administers over a period of weeks or months small intraperitoneal doses of a 0.1 per cent solution of trypan blue to full-grown rats. The palest hue of general color eventually characterizes such an animal, but this is not, of course, due to the slightest trace of dyestuff in the body-fluids or tissue-juices, but solely to the beginning accumulation of these minute, dense-blue crystals, at first exclusively in the macrophage cells. (See fig. 38.)

Protocol: Rat 325, injected intraperitoneally with a 0.1 per cent aqueous solution of trypan blue, February 13, 16, 19, 22, 25, and 28, 1 c. c. each day.

March 1: A faint blue shows at the base of the ears. Skin over the body is stained light blue. Subcutaneous tissues are light blue, deeper blue at the injection-point. Films from the abdomen show, under the low-power, sharp, bright-blue dye deposits in cells which are apparently macrophages.

Under the oil the macrophages are seen to be filled with bright-blue crystalline deposits. Vacuoles are present in these cells also and are stained often a light gray-blue, though some are without color. Macrophages from the thigh are similar to those from the abdomen. Neutral red stains the vacuoles and tinges parts of the blue crystals. Preparations so stained with neutral red (1:5,000) show a segregation-apparatus which is not greatly, if at all, increased over the normal in size or number of vacuoles (fig. 38). Janus green (1:10,000) stains numerous filiform mitochondria in the macrophages.

Under the oil, fibroblasts in films from the abdomen show a few dye deposits. These are minute granules, or vacuoles, and at times crystal-like deposits are present. Janus green stains normal mitochondria.

There are a number of these most perfectly segregated and crystallizable dyes, such as trypan blue, among which we may mention in particular two bright-blue dyestuffs from amido napthol disulphonic acids isomeric with the H acid, namely, the 1.8.3.5 and 1.8.2.4 acids. The 1.8.4.6 isomer (the so-called K acid) produces a dye whose traits in this respect are less marked. The experiments which we have just detailed are interesting in another direction, for they demonstrate that minute quantities of these quickly and perfectly segregated dyes are "fixed" by macrophages at such a rate that none of them remains in the tissue-juices to affect the fibroblasts, always slower in their reaction. It is thus possible, by means of an extremely low dosage with such dyes, to produce deposits sharply confined to the one cell type, just as it is invariably possible to satisfy oneself that one has affected the same cells alone, or to an overwhelmingly greater extent, by very concentrated acute dosage and the examination of tissue before the fibroblasts can react. Our attention was first directed to the phenomenon of minute crystal production in the macrophages by feeding experiments. Contrary to the assertion of Goldmann and others, it is not difficult to obtain a light general vital stain by the introduction of these dyes with food. So effectual, however, is the alimentary epithelial barrier that relatively enormous amounts of dye must be present to gain entrance by this route. We have achieved it by the employment of rather concentrated solutions of diffusible dyes in milk, or by the admixture of the actual dye powder with flour made into small biscuits. A microscopic examination of the intestinal tract in such animals shows an extensive cell necrosis of the epithelial investiture which is continually repaired through the abundant mitoses found in the necks of Lieberkuhn's crypts. It is our conviction that at no time is the efficacy of the epithelial barrier seriously impaired, but that minute amounts of the dye diffuse into the mucosal capillaries at these many injured points. (See figs. 39, 40, and 41.)

Protocol: Rat 4, fed Niagara blue BB (the benzidine homologue of trypan blue) from March 3 to March 26 (total of 5 to 6 grams of dye) in milk and egg mixture.

March 26: Animal is stained a light purple; abdominal skin is light blue. Low-power view shows tissue juices and fibers are free of any diffuse stain. Deep-blue discrete deposits are present.

Under the oil the deep-blue deposits are seen to be predominantly crystalline and are confined to the macrophages. The crystalline needles, or sheaves, are rather large structures (fig. 39).

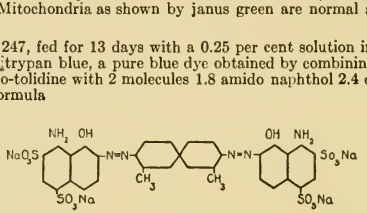
Protocol: Rat 100, fed by stomach tube with a suspension of Niagara blue BB in milk (1.5 to 3.0 per cent). November 27 to December 1, inclusive, 5 c. c.; December 2, 8 c. c.; December 3, 7 c. c.; December 4 and 5, 10 c. c. each day.

December 6: Blue color shows clearly in ears, though stain is not intense. Subcutaneous tissue shows no stain macroscopically.

Under the oil, macrophages appear as normally, except for the varying content of good-sized, highly refractive vacuoles resembling fat, between which lie bright-blue linear deposits. These are crystals and often lie in bundles of two or more. They are usually apparently free in the cytoplasm, but are also found in all stages relating them to the vacuoles, which in these cases have a brassy pale-red hue. Janus green shows normal filiform and granular mitochondria unrelated to the dye deposits.

Fibroblasts contain no blue dye. A few of the fat-like vacuoles, smaller than those in macrophages, are present. Mitochondria as shown by janus green are normal and unre ated to these vacuoles.

Protocol: Rat 247, fed for 13 days with a 0.25 per cent solution in milk of dye 1824, an isomer of trypan blue, a pure blue dye obtained by combining in alkaline solution 1 molecule o-tolidine with 2 molecules 1.8 amido naphthol 2.4 disulphonic acid, which has the formula



May 5: The abdominal skin, to the naked eye, can not be said to be stained.

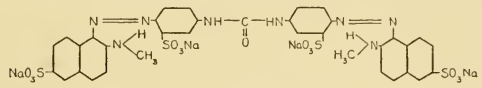
The oil-immersion shows that both types of cell possess an extremely scanty content of deep-indigo dye deposits which are almost invariably sharp rod-shaped and comma-shaped (figs. 40 and 41). In addition, in the macrophages and to some extent in the fibroblasts, there are present extremely faint blue spherical granules. It is worthy of note that the dense deposits of the vital dye in the macrophages are practically identical in morphology with the deposits in the fibroblasts, but in addition in the macrophages there has been a more general faint deposit of the dye in spherical granules of various sizes, some resembling those one sees in a deeply stained animal. They are, in fact, fairly uniform in size.

We were for a long time disposed to look upon the minute crystalline "dye bodies" produced in the macrophage connective-tissue cells by alimentary dosage as wholly unique and perhaps even related in some obscure way to metabolism. Nor did the secret of their method of production dawn upon us till long afterwards we undertook prolonged, exceedingly dilute administrations of dye by parenteral dosage—the intraperitoneal route. Dye deposits exclusively crystalline in form can also be produced in the fibroblast cells by dosages, still very dilute, but in excess of that required to affect the macrophages alone. In doses sufficient to effect such fibroblast deposits, the macrophage segregation-apparatus will always have been considerably enlarged, though within the vacuoles, crystals or bundles of crystals almost invariably occur, distorting the individual vacuoles into angular, polyhedral, diamond-like, or simple fan-shaped structures.

Crystals also occur in the cells of vitally-stained animals which have been treated in an entirely different manner with some of the vital azo dyes. We would designate these "saturation crystals." They are produced under conditions of very considerable dosage. The stain in these instances, instead of expressing its effect solely, or almost solely, in crystalline structures, as is the case with "low-dosage crystals," may establish a very extensive vacuolar segregation-apparatus; but, in

addition, at various points, either within the vacuoles or between them, crystalline condensation of the vital dye at length occurs. The "saturation crystals" may be produced typically in the case of vital dyes with which it is impossible to produce crystals with low dosages of the dye. In the case of the macrophages of animals treated with dye 245, the "saturation crystals" appear to "drain" the vacuoles of the entire segregation-apparatus of these overloaded cells, which are now colorless, and between which the characteristic long, deep-saffron needles of the vital dye appear. (See figs. 42, 43.)

Protocol: Rat 2286, injected intraperitoneally with 1 per cent solution of dye 245 (an isomer of vital new red) with the following formula:



May 16, 17, 19, 22, 25, 28, 31, June 3, 6, and 9, 1 c. c. each day; June 12, 0.8 c. c.

June 13: The animal is stained a brilliant red with some orange hue. Subcutaneous tissue of thigh is a deep fiery red, and even thin films are bright pink. Under the low-power one detects immediately macrophages as faint-pink masses containing deep-red linear deposits which, even at this power, are appreciated as crystals. One can not with certainty make out the fibroblasts.

The oil shows that the macrophages are entirely full of small vacuoles in which the color is extremely pale. Among these are straight, sharp, needle-like, deep eosin-colored crystals. These form complicated "brush-piles" in the case of macrophages along blood-vessels and are present to some extent in practically all of the macrophages (fig. 42).

The fibroblasts and their processes are full of fairly large, irregular-shaped, faint orange or yellow colored vacuoles. A small portion of these vacuoles have red crystals and needles related to them (fig. 43).

Still another set of circumstances may give a deposition of vital-dye crystals within these cells. We refer to the appearance of crystals in the case of the employment of a dye and a dosage which produces a vacuolar segregation-apparatus devoid of these structures, but which in decolorizing tends to concentrate its dye-content between the vacuoles in crystalline form. (Note the appearance of decolorization crystals in the case of dye T 148, rat 18–2, page 13 and rat 19–2, page 22, which appeared one month after dosage was stopped.)

4. Although Kiyono (1914) and Pappenheim and Nakano (1913) have reported "double-staining" experiments with acid and basic dyes, or with two acid dyes, it would appear that no one has made cytological studies of great accuracy in this field. In particular, the necessary accurate records on the actual effect of the first dye have not usually been secured from a study of skin samples taken under anesthesia before dosage with the second color is begun. The two vital dyes employed for such experiments must differ as widely as possible in their color, a crimson-red and a deep-blue dye giving admirable distinction. In two dyes which, when administered alone, produce approximately the same effect, *i. e.*, enter the cell at the same rate and are segregated in the same way, pronounced color mixture in the segregation-apparatus always occurs after dosage with the second compound. It is true that some vacuoles appear to carry only the first color and others clearly only the second, but deposits of uncontaminated color are rare. Such experiments

show in general that the places for the reception of these substances are not specific. The compounds in question, though differing in color, are indeed so near alike that it would be surprising if they were segregated in their manner of deposition in the cell. The phenomenon is, of course, entirely different from the metabolic segregation of substances occurring, for instance, in plant cells, where the plastids for the reception of starch do not contain chlorophyll, and *vice versa*.

Far more crucial are the experiments made with dye substances which differ not only in color, but also in the rate and form in which they are segregated in the cell. If the second substance is a crystallizing substance (like trypan blue), its employment as a second dye in such a series of experiments can be detected, not merely by blue contamination of some of the pre-existing red vacuoles, but also by the angular or seedlike distortions of these structures which its crystals provoke. (See figs. 44, 45, 46, 47, 48.)

Protocol: Rat 19–2, injected intraperitoneally with a 0.5 per cent solution of dye T 148, January 31, February 5, 10, and 15, 4 c. c. each day.

March 27: Subcutaneous tissue of right thigh examined. Animal stained a bright pink. Skin of thigh light pink. Under the low-power all cells are seen to carry pale-pink vacuoles, the macrophages being distinguished with certainty along the blood-vessels only, where concretions are more pronounced.

Under the oil, cell types can be differentiated. The macrophages contain watery vacuoles which show slightly more color than those in the fibroblasts and are more irregular in size and less closely packed. Neutral red 1:10,000 distinguishes the macrophages everywhere, because of the deep orange tint assumed by these vacuoles. In addition, much deeper staining concretions are present in many of the vacuoles (fig. 44).

The fibroblasts are distinguished by the possession of enormous numbers of quite uniform-sized deposits in which the color is only a pale yellow. With neutral red these assume a uniform pink, which is very much beneath the intensity of the vacuoles of the macrophages. Occasional instances of the characteristic rod-like crystals of this dye are seen. These do not appear to be related to vacuoles, but to lie between them.

Janus green gave a positive reaction for both types of cell. It is particularly interesting to see the mitochondrial apparatus in the fibroblasts which are already gorged with dye deposits. They are diffusely distributed throughout the protoplasm, little groups of them sometimes occurring where the dye deposits are relatively less abundant and also occasionally along very delicate strands of almost transparent protoplasm in which dye deposits are absent. The ease with which the mitochondria can be demonstrated in the macrophages where they are seen first is remarkable.

Same animal injected with a 0.5 per cent solution of trypan blue, March 28, April 1, 3, 7, 9, 13, 15, 18, 21, 24, 27, 30, May 3, 6, 9, 12, 14, 16, 18, 20, 22, 24, and 26, 1 c. c. each day.

May 27: Subcutaneous tissue from left thigh examined. Animal stained a deep blue. Subcutaneous tissue deep blue. The low-power shows that the macrophages alone have enough dye to be seen, but in them the dye is in the form of small, elongated, dense-blue deposits, so as to make brilliant display.

The oil shows the macrophage deposits to be almost exclusively crystalline. Neutral red 1:10,000 stains seed-shaped structures in the macrophages, where the blue content was previously very faint, and so intensifies the reaction of these cells (macrophage reaction) (fig. 45).

The fibroblasts remain full of very small, now almost colorless vacuoles produced by the previous dye treatment. A considerable number of the vacuoles, but by no means half of them, house a single minute blue crystal or, in rare instances, a sheaf of crystals of trypan blue. Several apparently free crystals are also found (fig. 46). With neutral red 1:10,000 the color in the fibroblasts is an extremely pale vermillion, so that it can not be seen with the low-power.

The case shows that there has been greater difficulty in the lodgment of the trypan blue in the fibroblasts than would be the case in a previously untreated animal, for there are fewer of these than we would otherwise see; furthermore, the blue crystals are minuter structures than would otherwise occur with this dosage. They are probably in all cases lodged in the T 148 vacuoles and

apparently can not increase as rapidly as would be the case were they formed *de novo* in the fibroblastic cytoplasm. Prolonged dosage of this case will, however, probably force them to break their vacuolar boundaries and grow, but even with this occurring they will still fail to occupy all of the vacuoles present, the tendency of trypan blue being here clearly proven to condense in fewer centers.

Same animal, given further treatment with a 1 per cent solution of trypan blue, May 29, 31, June 2, 4, 6, 8, 10, 12, 14, 16, 19, 21, 23, 25, 27, and 29, 1 c. c. each day.

July 9: Animal is stained a deep blue. Films taken from the middorsum, over the spinal musculature. They are deep blue macroscopically. Under the low-power brilliant blue dye deposits are seen in all macrophages. The elongated character of these seems evident even at this power. The fibroblasts are also invariably evident at the low power, carrying a paler and sparser blue content.

The oil shows that cell types are easily distinguished by the usual difference of cell morphology, as well as by the nature of the dye deposits. The macrophages have the majority, if not all, of their vacuoles, which are of an intermediate, fairly uniform size, converted into angular structures frequently elongated in only one dimension (spindle-shaped), but often multipolar. The very deep blue color is uniformly distributed in these vacuoles, although an accentuation along one edge (the crystals) can often be made out.

The fibroblasts, although occasionally having a few deposits as large as the average of those in the macrophages, have typically smaller, usually elongated ones. The larger accumulations have the angular character and the deeper boundaries or cross-structures existing within them that characterize the macrophage vacuoles and are to be interpreted as distorted vacuoles due to the overgrowth of crystals which have formed within them. Occasionally deep, small, linear deposits of trypan blue are connected by threadlike structures, and this would seem to indicate that the crystals may form on these structures. Neutral red shows that there are a good many T 148 vacuoles that still escape much contamination with the blue.

Protocol: Rat 53, injected intraperitoneally with 0.1 per cent solution of dye T 148, March 1 to 4, inclusive, 6, 7, 8, 10 to April 14, inclusive, 1 c. c. each day.

April 15: Subcutaneous tissue from thigh examined. Animal is stained a deep pink. Skin of thigh is bright pink. Under the low-power, both types of cell are seen to be filled with pale-pink vacuoles.

Under the oil, the fibroblasts have their cell-body processes outlined by the possession of immense numbers of small, faint-pink vacuoles, among which now and then the short, deep-red, crystalline rods characteristic of this dye are found. These are abundant in some cells. It is surprising that they may be entirely lacking in others. While the majority of the crystals are probably between the vacuoles, some are clearly connected with the vacuoles with which they have formed.

The macrophages are distinguished by the possession of uniform-sized, round, somewhat larger vacuoles with the usual occurrence of much larger ones. These cells contain very much more dye than the fibroblasts, judging by their general color, which is an emphatic pink, while the fibroblasts are merely tinged. The macrophages frequently contain in their vacuoles concrements of the dye. Neutral red 1:5,000 gives a striking macrophage reaction, staining the vacuoles of these cells a light brick-red, whereas the vacuoles of the fibroblasts are still pale in color. No crystals are found in the macrophages, unless we except an occasional small rod present in a vacuole.

It is evident that crystals have been produced by the saturation of the cell with dye.

The same animal was injected intraperitoneally with a 0.5 per cent solution of trypan blue, April 16 to 19, inclusive, and 21, 1 c. c. each day.

April 22: Autopsied. Animal is stained a deep blue. Subcutaneous tissues are deep blue with purplish tinge. Under the low-power there is a multiplicity of deposits in all cells and an evident bluing of most of the macrophages.

Under the oil, the fibroblasts have not stored appreciable amounts of blue dye. Short blue rods, almost invariably in vacuoles, are occasionally encountered. Infrequently there are a few larger vacuoles which contain blue crystals, and a scattered muddying by trypan blue of the T 148 vacuoles can be found on searching.

Macrophages are characterized by the contamination of a part of their vacuolar system by the blue dye, which, in addition to the diffuse coloration of vacuoles is found condensing, sickle-like, as deep-blue trypan crystals at the vacuoles' edges (fig. 47). Abdominal macrophages do not display these sickles, which are so frequent a phenomenon in the thigh and thorax, but contain chiefly pink, lilac, and purple vacuoles, in some of the largest of which are blue crystals, as well as red and blue concrements. Red concretions and crystals occur now and then in vacuoles in all parts studied (fig. 48).

The case is chiefly notable for lack of much blue in fibroblasts (indeed often not detectable at all) and for the crystals of trypan blue which have been able to form in T 148 vacuoles, the blue color of which is never very marked.

It is notable, then, that the trypan blue crystallizes out with the same readiness in the vacuolar system created by dye T 148 as would characterize it were the preceding red dye not present.

The cell's tendency to handle low doses of trypan blue and its biological homologues at relatively few places in the protoplasm is evidenced by the insignificant enlargement of the segregation-apparatus which, as we have already mentioned, such dosage produces. We have repeatedly employed, for the first treatment in our "double-staining" experiments, dye-stuffs which, either from their essential nature or from the dosage with which they are employed, produce an abundant and large vacuolar segregation-apparatus. Though this apparatus is consequently, as it were, waiting in all parts of the protoplasm to receive the blue secondary dye (dilute dosage with trypan-blue homologues), yet the latter does not avail itself of many more of these sites for anchorage that it would when employed in this dosage as the sole stain. It is apparent from this that the character and number of vital-dye deposits with any one method of dosage of a particular vital stain is not greatly influenced by the type of segregation-apparatus which already happens to have been produced in the cells treated. No data would appear to be more conclusively in favor of the view that "dye bodies" are dependent chiefly on the nature of the vital dye and on the exact method of its administration. Such experiments also furnish, it would appear, irrefutable evidence that even the conditions for crystallization are, for each dye substance, laws unto themselves, and that the tendency for the second dye to crystallize is not seriously interfered with through the fact that it must occupy vacuoles already filled with the first dye substance and apparently in more soluble form. These ideas, however, gained their most convincing demonstration by the production of analogous pictures when simultaneous dosage with an actual mixture of the same dyes was employed.

Protocol: Rat 2269, injected intraperitoneally with a mixture of equal parts of a 0.1 per cent solution of trypan blue and a 0.1 per cent solution of dye T 148 April 18 to 27, inclusive, 1 c. c. each day; April 28, 0.8 c. c.; April 29, 30, May 1, 3, 5, 7, 9, 11, and 13, 1 c. c. each day.

May 14: Films from right thigh examined. The animal is stained in general a light plum-color. Under the low-power the macrophages only are seen. They carry brilliant blue deposits, which, even at this power, can be identified as crystalline. The fibroblasts can be seen only with difficulty, but appear to display a pale-blue color.

Under the oil, the macrophages are seen to exhibit their trypan-blue deposits exclusively in the form of pale-blue, seed-shaped structures, sharply pointed at both ends, having a dense-blue single or double edge. Instances of a central deep-blue crystal, with or without lateral ones, are also seen. Cases of bodies which are almost spherical and possessing on one side a sickle-shaped condensation are also frequent. Instances of free, delicate, needle-shaped crystals, while seen after much search, are rare. Neutral red shows only a small number of other vacuoles. Janus green (1:10,000, four minutes) stains normal mitochondria.

The fibroblasts are full of pale-yellow, irregular-shaped T 148 vacuoles which fill the protoplasm, including its chief processes. In a portion of these (always a half dozen instances) the trypan blue has accumulated so as to give a marked blue hue and in some cases to produce a minute blue crystal. Instances of these minute crystals unrelated to the T 148 deposits are not seen, and the blue, when most emphatic, is so difficult to see that the case must be continued for at least another

20 days. One would predict that the trypan-blue deposits in the fibroblasts will eventually form sturdy crystals betraying no sign of their origin from vacuoles. It is to be emphasized that the belief that these have all arisen from vacuoles in which both dyes are present is due to the impossibility of deciding whether a great many of the vacuoles have or have not a small amount of blue in them, the morphology of the vacuolar system being the same whether blue is or is not present. Janus green shows typical filiform mitochondria.

The same animal was injected further with a mixture of equal parts of a 0.1 per cent solution of T 148 and a 0.1 per cent solution of trypan blue, May 16, 18, 20, 22, 24, 26, 28, 30, to June 1 to 17, inclusive, 1 c. c. each day; June 18, 0.6 c. c.; June 19 to 29, inclusive, 1 c. c. each day.

July 16: Films from left thigh examined. Animal is stained a light violet-color. Skin of left thigh light blue. Under the low-power, minute deep blue, apparently crystalline deposits are seen in some cells, persumably macrophages.

The oil confirms this. Not much change in the cell picture recorded May 14 has resulted. The macrophages still, as a rule, possess their trypan-blue deposits in the form of small, intravacuolar crystals. Free needles of a larger size are present in many cells, especially along blood-vessels. Neutral red shows that the small vacuoles are numerous in macrophages and the tendency to neutral red concretions marked.

Fibroblasts are packed with T 148 vacuoles and exhibit no departure from the condition on May 14, except that it is usual to find one or two trypan-blue deposits of deep blue, either seed-shaped or needle-like, and unrelated to T 148 deposits. These are apparently the "sturdy crystals" looked for. It is to be emphasized that there is no noticeable increase in the number of T 148 bodies which have been chosen for trypan-blue deposits, *i. e.*, half a dozen only house the blue dye, but the blue is now in crystalline form within or along the edge of vacuoles and also apparently free.

We have also carried out experiments quite the reverse of those just cited. We have employed dyes which create a large vacuolar segregation-apparatus after the preliminary production of small pure crystalline deposits with trypan-blue isomers. While some few of the original crystalline deposits happen never, subsequently, to be enveloped by the vacuoles created by the second dosage, most of them are rapidly inclosed in this way and with continued further dosage with the second dye are eventually dissolved in its vacuolar fluid. It could be expected, and, indeed, it has invariably resulted in all experiments of this type, that the main mass of the large segregation-apparatus produced by the second dye contains no color contamination with the dye first employed. A simple explanation for this is at hand in the fact that most of these structures are *de novo* formations, originating only after the inauguration of the second dye treatment. We have, in such experiments, scanty "dye bodies" of pure color of the first dye, many of pure color of the second vital dyestuff employed, and a considerable number of mixed "dye bodies" mingled with them. (See figs. 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64.)

Protocol: Rat 16, injected intraperitoneally with a 0.5 per cent solution of trypan blue, January 30, February 2, 5, 8, 11, 14, 17, 20, 23, 26, March 1, 4, 10, 13, 16, 19, 22, 25, and 28, 1 c. c. each day.

March 30: Films from right thigh examined. Animal is stained a bright blue. Under the low-power, deep-blue dye deposits are present in both types of cell. Some macrophages can be distinguished by their larger dye deposits. Fibroblasts are seen to be heavily laden with deposits to the full extent of their processes.

Under the oil, the macrophages appear to have even less dye than the fibroblasts. Their dye content varies from one or two blue vacuoles containing crystals to great numbers of such. Where much blue dye is present deep blue crystals lie free in the cytoplasm between vacuoles (fig. 50). Pale blue and colorless vacuoles are numerous in macrophages containing but little blue dye. Neutral red gives a specific stain in the macrophages. Apparently pure red vacuoles are very numerous in many of these cells, which show relatively little blue. The large vacuoles containing blue crystals are stained by neutral red. Some blue crystals are present unaffected by the neutral red and lie free in the cytoplasm.

The fibroblasts are distinguished by the possession of great numbers of deep-blue crystalline deposits, among which are small, angular and filar neutral-red bodies. The typical cell contains at least as many neutral-red as blue deposits. Their morphology is almost exclusively seed-shaped and linear, though some instances of small, almost round, though angular, deposits are seen. Large crystalline masses are not uncommon, as shown in the drawing (fig. 49).

In conclusion, it is to be emphasized that almost all of the deposits in the most frequently occurring cell are crystalline in nature, whether they be deep blue or only orange from neutral red, and they are almost invariably quite delicate structures.

Same animal was injected with a 0.1 per cent solution of dye T 148, March 30 to April 14, inclusive, 1 c. c. each day.

April 15: Subcutaneous tissue was examined from left thigh. The low-power view shows deep-blue deposits in all cells.

The fibroblasts do not appear to have changed significantly from the last autopsy date as far as their blue crystalline deposits are concerned. They still contain remarkably sturdy, deep-blue, purely crystalline structures, with now and then the occurrence of a single deep-blue vacuole, which, however, often shows some angular distortion. These cells are, in addition, full of pale-yellow T 148 bodies, spherical or angular and of fairly uniform size (fig. 52).

The macrophages are on the whole remarkably small cells filled with vacuoles, the great majority of which are pink. Small fat vacuoles are numerous. These cells lie in colonies, as if cell division had taken place. They contain deep-blue deposits, usually in the form of short, straight crystals included within vacuoles which are either pale pink or pale blue. The amount of deep blue in the macrophages is extremely variable as one goes from one cell to another. A few contain red vacuoles only. Many contain but one vacuole filled with deep-blue crystals and often much larger than the typical T 148 vacuoles (the majority of which are about the size of mast-cell granules) (fig. 51). Macrophages containing a great quantity of deep blue are rather rare. Pale-blue crystals are so infrequent in these cells as to be negligible, although in the cells containing a great quantity of blue dye many deposits show no pink and preserve a crystalline nature, though usually connected with deep-blue vacuoles.

On the whole, the picture is identical with that found before, as far as the blue deposits are concerned, in the fibroblasts. The T 148 has formed vacuolar deposits which take the place of and are more numerous than the round and linear neutral-red bodies described before, but the deep-blue (crystalline) deposits seen before are not supplanted or even inclosed by the T 148 vacuoles.

Same animal injected with a 0.5 per cent solution of dye T 148, April 15 to 29, inclusive, May 1, 3, 5, 7, 9, 11, 1 c. c. each day.

May 13: Examined subcutaneous tissue from abdomen. Animal stained a deep purplish blue. Subcutaneous tissue looks bright blue to the naked eye.

Under the oil, cell differentiation is easy. The macrophages are undoubtedly chiefly responsible for the purple color, their abundant intermediate-sized vacuoles being usually pale heliotrope in color, the larger ones tending toward blue. No instances of crystals can now be seen, but deep-blue masses exist in these larger vacuoles as well as in some of the smaller ones.

The fibroblasts now give the impression of being stained with dye T 148 rather than with trypan blue. Their cytoplasm is gorged with dye T 148 vacuoles which have a pale pinkish-yellow color. A great many fibroblasts, however (almost half of them) contain 1 or 2 deep pure blue, seed-shaped trypan deposits, and in some cells the number of these bodies may reach 15 or 20. Some films show practically all of the fibroblasts as elliptical, rounded-up elements, with their protoplasm full of great numbers of entangled pale yellowish-pink "threads." These cells are not different in their trypan blue deposits from others, *i. e.*, perhaps half of them are free from trypan-blue deposits. A great number have 2 or 3 such deposits, and others a considerable content (15 to 25).

The persisting deep-blue color of the animal shows that much trypan blue is still present, and the microscopic picture shows that most of the dye is present in the macrophage cells, where its contamination, however, with the subsequent red-dye treatment allows no pure-blue deposits to be seen. The majority of the fibroblastic blue deposits have disappeared, but those persisting are still pure deep blue and are the only uncontaminated deposits now present in the skin. It is likely that continued T 148 dosage will give great numbers of entangled threads in fibroblasts all over the body; they are here very abundant over the whole abdomen.

Injection of a 0.5 per cent solution of dye T 148 continued, May 14, 17, 20, 23, 27, 31, June 2, 4, 6, 8, 10, 12, 14, 16, 19, 21, 23, 25, 27, and 29, 1 c. c. each day.

July 17: Autopsied. Animal is stained a reddish violet or heliotrope. Abdominal skin is reddish heliotrope. The low-power view shows that vital-dye deposits are so lacking in color as to be barely capable of detection, all cells being faint pink.

The oil confirms this and shows at once the great development and extent of fibroblasts whose protoplasm about the nucleus and in all processes, many of which are long, is closely filled with uniform-sized, refractive, pale-yellow, various-shaped vacuoles, among which and in frequent connection with which are "thread" structures. These linear structures may in some cells be the predominant feature, the cell-body being rounded up and the "threads" lying in great numbers close together, taking usually one general direction. The fibroblasts frequently (perhaps a quarter of all cells) contain from 1 to 4 or 5 seed-shaped deep-blue bodies, evidently trypan-blue crystal remains.

The macrophages are usually small round elements with medium-sized uniform vacuoles. Only after much search are trypan-blue deposits (not infrequent in the fibroblasts) found, and then these are single and minute. The vacuoles react somewhat more intensely with neutral red than the fibroblast vacuoles.

Films from the back (over erector spinæ) show few fibroblasts with "threads." Occasionally fibroblasts are found with red dye T 184 rodlets, and very rarely these are abundant. In such cases they are sharply separate from the few trypan-blue deposits present. Dye T 148 rods are almost always free from vacuoles, but all stages of their formation in vacuoles may be seen. They are not present in macrophages.

The skin of the scrotum contains the same types of deposits. "Threads" are not very abundant.

Protocol: Rat 43, injected intraperitoneally with a 0.1 per cent solution of trypan blue, March 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, April 3, 7, 9, 13, and 15, 1 c. c. each day.

April 18: Subcutaneous tissue of thigh examined. Animal is stained a uniform light robin's egg blue. Skin of thigh same color. Under the low-power, one sees chiefly bright-blue deposits in the macrophages, but also trails of scantier and smaller deposits belonging to the fibroblasts can be made out. At this power, however, the predominance of the macrophages is so marked as to give an almost exclusive "macrophage reaction."

Under the oil, macrophages contain many deep-blue crystals and dark or light blue angular structures (distorted vacuoles). No true blue vacuoles are found. The crystals vary in their length and thickness. In some cells they are very long and delicate. With neutral red there are instances of round bodies in all the macrophages and an abundance of them in some few macrophages, but practically all of the trypan blue present is in the form of crystals, usually deep blue, though light blue ones are present.

The fibroblasts contain a scanty, but pretty constant, content of small angular deposits which would be identified as minute crystals. The neutral red also shows a number of pale vermillion linear and angular bodies identical in morphology with the blue ones, but invariably paler in blue content. They range down to very small bodies, not much above the limit of visibility. They are about as abundant in number as the deep-blue bodies, but the total of both structures does not make any great number of deposits for these cells (fig. 53).

Same animal injected with 0.5 per cent dye T 148, April 18, 0.5 c. c.; April 19 to 28, 1 c. c. each day.

April 30: Autopsied. Animal is stained a plum color. Subcutaneous tissue is purple. Under the low-power deep-blue deposits are seen in macrophages. The red deposits are so faint that little of their nature can be made out.

Under the oil, both types of cells are well laden with dye T 148 vacuoles. The fibroblasts especially are filled with them.

Macrophages carry deep-blue, sturdy, crystalline deposits which, while tending to occur in pairs or forked, are not greatly influenced by the dye T 148. Crystals are found in vacuoles, on searching, but in the majority of cells they are not inclosed in vacuoles, though these vacuoles (dye T 148) are very abundant. Where the trypan-blue crystals are most numerous and long the dye T 148 vacuoles are small. Trypan-blue crystals are usually substantial, but are occasionally more needle-like, and in these instances the dye T 148 vacuoles are smaller (fig. 57). Near the puncture-point some of the dye T 148 vacuoles attain greater size, and within these there are a number of needles. The tendency to the formation of vacuoles about crystals is much more expressed on the abdomen, and one finds here not infrequently cells with deep-blue vacuoles, however, not yet perfectly spherical, and in which one or two crystals are still evident (fig. 54). This is more frequently the case in the mesentery.

Fibroblasts are packed with very uniform, fairly large vacuoles with a pale yellow color, slightly refractive. A limited number of small, deep-blue crystals invariably occurs, and on comparison with the previous drawing it is evident that but a portion of the trypan-blue deposits seen before are now detectable. Perhaps the majority of needles are inclosed in dye T 148 vacuoles, the remainder being free (figs. 55, 56).

Protocol: Rat 15, injected intraperitorneally with a 0.5 per cent solution of trypan blue, January 30, February 3, 7, 11, 15, 19, 23, 27, March 3, 7, 11, 15, 1 c. c. each day.

March 18: Subcutaneous tissue from right thigh examined. Animal is stained a deep blue. Subcutaneous tissues are deep blue. Under the low-power, macrophages appear in sharp contrast to fibroblasts on account of their greater dye-content, although the fibroblasts contain sufficient dye to enable one to outline them under this power.

Under the oil, macrophages are filled with deep-blue deposits which are most often of the crystal-vacuole morphology. Blue crystals are also present free in the cytoplasm, some of them very light blue. The crystals in vacuoles usually distort them, protruding out for some distance. Neutral red stains the dye vacuoles and modifies the blue color in many of the naked crystals also. It stains some small, round and triangular vacuoles overlooked before between the larger dye vacuoles (fig. 58).

Fibroblasts contain numerous deposits which are entirely linear, much more delicate than the free crystals in macrophages. There are deep and pale blue crystals of uniform length and thickness. A few fibroblasts may be found with long threads attached to deep-blue deposits and themselves stained faintly. Neutral red stains some small seed-shaped vacuoles not seen before and also stains the paler blue deposits (fig. 59). Janus green shows normal mitochondria in both types of cells.

Same animal was injected with a 1 per cent solution of dye T 148 intraperitoneally, March 18, 4 c. c.; March 20, 2 c. c.

March 22: Subcutaneous tissue from left thigh examined. Animal stained a heliotrope. Under the low-power the skin from the thigh shows many deposits both of blue and red in all cells. Only those macrophages which show large vacuoles can be identified with certainty.

Under the oil the macrophages are characterized by an abundance of practically uniform-sized round vacuoles, a few of which have blue in them. There are almost invariably a very few larger deep-purple vacuoles containing either amorphous matter or crystals. Some of the smaller vacuoles contain a crystal each. Neutral red gives a somewhat more intense color to the vacuolar system of these cells than with the fibroblasts (fig. 60).

The fibroblasts are characterized by the lack of much, if any, disturbance with their crystalline trypan-blue deposits. These are usually free in the cytoplasm, although occasionally a pink vacuole can be seen about one. Amongst them are fair-sized, angular, yellow dye T 148 deposits which are on the average slightly smaller than the customary slightly round deposits in the macrophages. Neutral red shows nothing new except an occasional tail to the angular dye T 148 deposits (fig. 61).

Protocol: Rat 222, injected intraperitoneally with a 0.5 per cent solution of trypan blue. February 25 to March 4, inclusive, 1 c. c. each day.

March 17: Examined subcutaneous tissue from thigh. Animal is stained a bright blue. Subcutaneous tissue is bright blue. Under the low-power, cell types are distinct. Macrophages contain an abundance of deep-blue deposits, while fibroblast deposits can barely be seen.

The oil shows that macrophages contain long blue crystals, free in the cytoplasm for the most part, but occasionally lying within and connected with blue vacuoles. Neutral red stains small vacuoles which have no vital-dye content. It also stains the large vacuoles with crystals and tinges parts of the blue crystals. Neutral red gradually replaces the blue, even on the more deeply stained crystals (fig. 62).

Fibroblasts contain crystalline deposits which are much smaller than those in macrophages and not as numerous. Neutral red shows great numbers of small vacuoles in these cells. It also stains many of the crystals.

Same rat was injected with a 1 per cent solution of dye T 148, March 17, 4 c. c.

March 19: Subcutaneous tissue of opposite thigh and of abdomen examined. Animal is a dusky reddish violet. Skin of thigh same. Low-power shows macrophages easily evident from their abundance of medium-sized, uniform light-pink vacuoles which crowd the cytoplasm.

The majority of the macrophages have lost much of their blue deposits and some all of their blue, unless we count a slight bluish tinge in some of the vacuoles. Many instances of small crystals surrounded by a pink vacuole are seen, and these may be interpreted as the most persistent crystals in the cell, the others having been completely dissolved by the vacuole which takes their place. Where the blue was previously in a large, dense vacuole this remains with no change except a violet blue from some T 148 contamination. Many cases are seen of cells which contain but two or three blue vacuoles, while the remainder are pink. Along blood-vessels in the thigh the blue crystals are apparently unaffected, but there are in addition in these cells a considerable number of small pink vacuoles (fig. 63).

Fibroblasts are distinguished chiefly by the fact that T 148 has not interfered with the delicate deep-blue crystals described before. These cells always contain, however, a considerable number of large pink dye T 148 vacuoles. Instances of vacuoles surrounding crystals, as is usual in macrophages, are rare, though this phenomenon has begun to occur (fig. 64).

EVIDENCE OF THE SIMILARITY IN NATURE OF THE FIBROBLAST AND MACROPHAGE "DYE BODIES."

The peculiar behavior of the fiber-forming cells of the connective tissue toward the vital acid dyes could hardly have escaped the notice of any acute observer working with these compounds. Goldmann, whose beautiful and extensive researches must be regarded as preliminary exploratory efforts of classic importance, concerned himself only with the predominant reactions secured by these dyes upon cells under various normal and pathological conditions. With the establishment of the conception of "pyroll cells" (our macrophages), and with his demonstration of the participation of these cells in various reactive processes, his work will have abiding worth, regardless of the lack of exact cytological scrutiny at many points. In the preliminary publication of our larger work (Evans and Schulemann, 1914), we indicated clearly that the fibroblasts enjoy a distinctive reaction to the vital stain. This fact has since been investigated with varying degrees of precision from two sources which have furnished several publications each—from the laboratories of Aschoff and Maximow. From the pathological department of Freiburg, and, especially, in the work of Kiyono (1914), has come an admirable critical reworking of most of Goldmann's field, but with improved discrimination as to cell types. From the Petrograd laboratory, to which we are indebted for fundamental, clear-cut histological work, especially on the areolar-tissue cell types, we have the papers of Tschaschin (1912, 1913) and his astonishing interpretation of the mitochondrial nature of the dye deposits. If the facts concerning the macrophage stain which we have carefully enumerated in this paper furnish, as we believe they do, a convincing refutation of this viewpoint so far as it concerns the great phagocytic cells, it will be of importance for us to ascertain what light the same type of experiments can shed on the nature of the somewhat different reaction to the vital stains displayed by the fibroblast cells. Tschaschin's opinion on the mitochondrial origin of the macrophage stain was admittedly weak in proof and would appear not to have been taken seriously by most subsequent writers. It was influenced confessedly by the conditions which he believed obtained in the fibroblasts. But in the last publication from Maximow's hand (1916), Tschaschin's teacher has been able to show a practically normal mitochondrial apparatus, even in the phagocytic cells, merely by a more successful employment of iron-hematoxylin stains on Zenker-formol material, and he himself has at least seriously weakened his pupil's view that the mitochondrial apparatus in these cells is wholly devoted to that granular and vacuolar transformation which Tschaschin looked upon as occurring for the sake of storage of the vital dye. We have already instanced our profound distrust of the specificity of fixed staining methods for the mitochondria of these cells, and our success in the elective display of the mitochondria, even of

badly overloaded "polyblastic" cells, by means of the method of supravital staining after sufficiently patient empirical efforts with various concentrations of janus green B.[1]

It is in the fibroblasts, however, that Tschaschin has announced his discovery of an elegant and purely elective tingeing of the mitochondrial apparatus of the living cell by means of the vital azo dyes, and even those observers who feel compelled to view with skepticism his stand on the macrophage question have been inclined to accept or have refrained from criticism of his theory of the mitochondrial identity of the "dye bodies" in the fibroblastic cells (*cf.* especially Kiyono). Aside from the fact that no such affinity between mitochondria and the azo dyes can be demonstrated in any other cells or tissues, Tschaschin's interpretation is fortunately open to direct examination by specific supravital staining of the mitochondria, such as we have carried out in the macrophage cell. We can not resist again the opportunity to remark on the inadequacy of other agents for the *elective* display of the mitochondria. Neither the hematoxylin, the alizarine, nor the fuchsin methods will accomplish this end, for with all three of them the segregation-apparatus, whether in granular or vacuolar form, is, to varying degrees, tinged. These facts, in our estimation, have led Maximow in his latest tissue-culture studies (1916), to trace erroneously a connection between these two cytoplasmic structures. Our own position is wholly that taken by the Lewises, who, after extensive cytological analyses of the same material (cultures), are positive in their statements of the possibility of precise separation of the vacuolar and mitochrondrial elements by supravital tinctorial methods (neutral red, Nile blue B, brilliant cresyl blue, janus green). Indeed, no other methods than the last will bring conviction here, for neither criteria of size, distribution, or morphology are anything but confusing in precisely this field. We must confess that the behavior of the arcolar fibroblasts of the rat towards trypan blue and some of its isomers gives us dye bodies which with striking versimilitude reproduce the mitochondrial pictures obtained by most methods; but one of us (Scott, 1915) has already reported the successful demonstration of the true mitochondrial system in such fibroblasts stained to varying degrees with isamine or trypan blue, and where the azo-dye bodies resemble closely mitochondria and the figures supplied by Tschaschin. Even in the case of normal tissue, the descriptions of Dubreuil (1911–1913), Maximow (1916), and others of the mitochondrial apparatus of fibroblasts are, none of them, quite accurate on account of the lack of specificity of their methods and consequently of the inclusion of other cytoplasmic structures, even though these are not very abundant in fibroblastic cells. For this reason it is perhaps not unessential that we present carefully executed drawings of the normal appearance of fibroblast mitochondria, carried out by the supravital method on living cells (figs. 65 and 66).

The circumstances which hinder a supravital demonstration of mitochondria in the macrophages of animals deeply stained with azo dyes operate to defeat our success with fibroblasts, for the tissue-juices, charged with the acid dye,

[1] With which we would place, in addition to the other janus dyes investigated by Cowdry, methylene violet B A (Hoechst) and amethyst violet (B. A. S. F.)

tend to combine with the basic dye (janus green 1:100,000 to 1:10,000). The simple scheme of the employment of higher concentrations of the mitochondrial dye (janus green 1:5,000) over a long period of time (2 to 5 minutes) has sufficed to accomplish in these acid-dye-soaked cells a specific result. But the janus reaction can be secured in its usual specific way and by more usual methods if, after the attainment of the vital-azo stain, a considerable time is allowed to elapse so that the azo compound is not present in appreciable quantities, except in the actual segregation-apparatus of the stained cells. By either of these two means normal mitochondria can be demonstrated after the most various dosage and with the most various dyes of the acid-azo series (figs. 5, 16, 37, 67, 69, 70, 71). In spite of the striking similarity in morphology, number, and distribution of the vital "dye bodies" and the mitochondria in fibroblast cells, we rest our ability to discriminate between these structures on repeated experiments with the living cells which may be summarized as follows:

SEGREGATION APPARATUS.	MITOCHONDRIAL APPARATUS.
The segregation-apparatus is electively stained by freshly made isotonic solutions of neutral red 1:10,000 and other basic dyes.	Mitochondria are not electively stained by any concentration of neutral red.
The segregation-apparatus is not stained by concentrations of janus green B which are elective for mitochondria.	Mitochondria are electively stained by appropriate concentrations of janus green B in isotonic salt (1:100,000 to 1:10,000, and under certain conditions 1:5,000).
When the segregation-apparatus is stained by solutions of janus green in excess of strengths specific for mitochondria, the stain remains, except for partial reduction, for a more considerable interval of time.	Specific janus-green stains of the mitochondria fade rapidly and completely after a short interval, in which the dye is reduced to a pink form (diethylsafranin).
The morphology of this system does not change on long standing; preparations 24 hours old are often not appreciably changed.	The morphology of this system changes conspicuously within several minutes, segmentation, swelling, or dissolution effects supervening.

It is interesting that in some instances, after extensive treatment with the acid-azo dyes, the mitochondria of fibroblasts occasionally occupy distinct cytoplasmic zones rather free from dye deposits (fig. 70). Usually, however, the mitochondria are not greatly disturbed in their arrangement, tending to be diffusely scattered, except for the invariable presence of some of them near the nuclear membrane; and this is true even with the employment of those vital dyestuffs which we have discovered to create a large segregation-apparatus in these cells. In the case of animals 19–1 and 24–1 (figs. 71 and 5), the mitochondria of the fibroblasts are electively displayed amongst the characteristic numerous large vacuoles created by dye T 148. It is apparent, then, that most of the dye deposits in the fibroblasts are not mitochondrial in nature, even though they have special characteristics (minute size and form) which would justify all but the most critical observation in aligning them with mitochondrial forms.

We desire to refer briefly now to an actual tingeing of the mitochondria of fibroblasts with acid-azo dyestuffs, in cases where the vital dye is in considerable concentrations. We have been able to satisfy ourselves, in cases of high intraperitoneal dosages, of the existence of fibroblasts in the areolar tissue of the abdomen, where the mitochondria are clearly but faintly tinged with the vital dye. This is probably a transient effect. It may be also a necrobiotic one, for other cells

in the same neighborhood show toxic effects of the dye (*e. g.*, nuclear staining). We have seen no evidence of the participation of these tinged mitochondria in the formation of the granular or vacuolar deposits of dye. Levi (1916) has reported mitochondrial tingeing in the case of fibroblasts of tissue cultures cultivated in pyroll blue plasma and jumps to the conclusion that he has verified Tschaschin's claim that the fibroblastic dye bodies are mitochondria. Before Levi's paper appeared, similar results in tissue cultures were repeatedly secured by M. R. Lewis, and we have had the privilege of seeing these indisputable preparations. This evidence does not, in our estimation, establish even the mitochondrial origin of fibroblastic dye deposits. It certainly could not enable one to declare that the usual dye bodies shown by fibroblasts, after treatment with these dyes, represent an electively stained mitochondrial apparatus.

We have previously refrained from describing still other characteristics of the kind of deposits which are frequently produced in these cells and which are, even more strikingly, so like mitochondria as to further justify this confusion. We refer to the greater tendency of the fibroblast to exhibit linear forms in its segregation apparatus, due either to the minute rod-like and needle-like crystals which form in these cells or to the production of characteristic "thread bodies," which we will describe later, but which, by all stages of transition, can be seen to belong to the segregation-apparatus and not to the mitochondria of these cells. The description of these phenomena, which are highly characteristic for the fibroblasts, must be deferred until we have established the *nature* of the "dye bodies" in fibroblastic cells by the same four lines of evidence which we have employed in the case of the macrophages and which speak even more conclusively for the dye-storage hypothesis of the "vital bodies" present in fibroblasts, though this deposition is greatly modified by the characteristic physiology of these particular cells.

1. A normal minute vacuolar segregation-apparatus is not lacking in fibroblastic cells (fig. 13). It is distinguished, however, by its insignificant development, shown by the small size and number of vacuoles when compared with those always present in the macrophages, and by the greatly lessened reaction of those bodies to supravital neutral red (1:10,000). In fact, only by the employment of neutral red can the almost glassy-clear protoplasm of normal fibroblasts be made to exhibit its low content (5 to 25) of the minute, punctate, granular, or small vacuolar structures for which we regard this dyestuff (in harmony with Dubreuil, Renaut, and M. R. and W. H. Lewis) as a specific test. This inconsiderable segregation-apparatus of the fibroblasts can be greatly modified by various methods of staining with acid-azo dyes. The greater variability in the extent of the apparatus normally characterizing the macrophages might tend to make some of our assertions about the exact dependence of the morphology of this system on the amount of dye dosage less certain than need be the case with fibroblasts; for it is possible in the fibroblasts to obtain a most complete array of quantitative data. Although we have described the usual scanty content of these cells in "dye bodies," the number and size of the latter speedily increase with increased dye dosage in the

employment of any azo dye and show characteristic differences, depending upon the type of dye used. This evidence is furnished in the most striking way by the behavior of fibroblasts after dosage with what we have called "negative," or non-diffusible, vital dyes. After subcutaneous injections with any of the large existing series of such compounds, the fibroblasts several centimeters removed from the injection site accumulate only the minute fibroblastic "dye bodies," which we in our paper with Schulemann and in common with Kiyono, Tschaschin, and others, have already described (fig. 33). Such is not the case, however, with the fibroblasts in the immediate vicinity of the site of injection and is never the case with fibroblasts at the actual point of injection. In all such locations, where much more dye substance has had access to the fiber-forming cells, the latter ingest greater quantities of the dye, never as great, it is true, as the macrophages in the same location, but so great that one may meet over a considerable territory fibroblasts whose protoplasm is completely filled with a vacuolar segregation-apparatus which is developed in direct response to the need of housing the vital dye (see fig. 72).

Protocol: Rat 200, injected subcutaneously with a 1.5 per cent solution of isamine blue March 15, 19, 22, 26, 29, April 2, 5, 9, and 12, 1917, 1 c. c. each day.

1918, March 21: Examined subcutaneous tissue from back (area of injection). Animal is stained a light greenish blue over the back. Subcutaneous tissue is bright blue in this area. Under the low-power, cells showing a high content of light-blue dye deposits are seen, but cell types are not distinct.

The oil shows the same difficulty in identifying cell types. The majority of fibroblasts are large cells, sometimes with two nuclei, whose cytoplasm is crowded with small, bright-blue deposits rather uniform in size and tending to be angular. Neutral red stains these vacuoles and discloses no new structures. The extent of cell processes filled with dye deposits is often very great, and apparently there is little or no cytoplasm free from dye (fig. 72).

Macrophages can be identified by their general morphology (shape of cell, size of nucleus, etc.) rather than by any peculiarity of their dye deposits, since these are very similar in size and number to those of the fibroblasts, though more often round. Near blood-vessels the macrophages are full of deep-blue vacuoles, some of which are large. In areas away from the injection-point these cells have vacuoles which are smaller on the whole and much fainter than those in fibroblasts and do not fill the cells as completely.

The fibroblasts of the serous membranes in cases of large and long-continued peritoneal injections, even with the negative as well as the positive azo dyes, show this great multiplicity of "dye bodies" in a most striking way.

Just as the fibroblasts in the case of the subcutaneous injection of a negative dye show great differences in their segregation-apparatus in strict accordance with their distances from the injection-point, so also do these cells exhibit more accurately than do the macrophages characteristic differences in the form and extent of the apparatus, depending on the physical properties of the particular vital dye. Compare, for instance, the typical trypan-blue deposits in the fibroblasts shown in figure 59 with the typical dye T 148 deposits depicted in figure 82. In the investigation of a long series of azo compounds it has been possible to discover dyes whose facility in entering the fibroblastic protoplasm is so great as to establish here an extensive vacuolar segregation-apparatus which has few or none of the peculiarities (minute size, irregular, angular, and filiform morphology) which with most vital dyestuffs characterize these cells. The discovery of dyes

which would affect the fibroblasts in this way was a particular boon to our investigation of the mitochondrial question as far as it concerned the fibroblastic cells, for the morphology of the dye deposits in such cases does not resemble remotely the mitochondria. Some of the most satisfactory of these dyes were red in color, thus affording chromatic distinction between them and mitochondria tinged blue with janus green.

2. The data which we have given for macrophages on the phenomenon of metachromasia and the proof it furnishes of the accumulation and flocculation of the vital dye have all been found to hold in the case of the fibroblasts. Indeed, it is a general rule that the accumulation, flocculation, or concrement formation, just as is the case with crystals, is easier to produce in the fibroblasts, providing our dosage is sufficient to get the dye into these cells.

3. The question of whether or not the "dye bodies" represent preformed cell-organs or are merely true accumulations of the dye, gains a convincing answer in the case of the fibroblasts, just as it did for the macrophages, by the production of pure dye-crystals in these cells. These are produced, as in the macrophages, by long-continued, greatly diluted dosage only with certain dyes (trypan blue and its isomers). Evident increased permeability of the macrophages, when compared with the fibroblasts, makes it necessary to employ only extremely dilute solutions of the azo dyes if we would effect pure crystal pictures in the great phagocytic cells. Such experiments, as we have already indicated, give us in fact an *elective* crystal deposition in the macrophages. But if slightly higher dosages are employed, both cell types in the skin are affected, the fibroblasts by pure crystalline deposits, the macrophages by crystalline and colloidal deposits about equally abundant; and by the continuation of such dosages the number of crystalline deposits in the fibroblasts can be greatly increased (fig. 73, 74, 75, 76).

Protocol: Rat 46, injected intraperitoneally with a 1 per cent solution of trypan blue, March 8, 11, 14, 18, 20, 23, 26, 29, April 1, 4, 7, 10, 13, 16, 19, 22, 25, and 28, 1 c. c. each day.

May 1: Animal is stained a profound blue. Under the low-power, both types of cell are heavily laden with deposits which seem, even at this power, wholly crystalline. Cell types are magnificently differentiated. Macrophages are blue-black, except for the nuclear area. They often lie in small colonies or chains of cells. Fibroblasts are a lighter shade of blue and the typical cell outline with its delicate processes is accurately preserved by the dye deposits which fill them.

Under the oil, macrophages usually have broad, blunt crystal masses of uniform size. Some cells contain crystals of longer, more delicate form. Neutral red tinges the broad structures and in some gives a picture of red, angular vacuoles with blue crystals distorting and protruding from them. As a rule, the crystalline deposition has advanced so far that large, uniform-sized, triangular and diamond-shaped, dense-blue masses, defeating an analysis, are the result. Some smaller rods lie between the large, blunt structures (fig. 75).

Fibroblasts have more delicate and longer crystals. At times these occur in groups and suggest that they have distorted a vacuole. Neutral red stains the vacuolar portion first. It shows practically no new structures. Threads do not occur in the fibroblasts (fig. 74).

Protocol: Rat 31–3, injected intraperitoneally with a 1 per cent solution of trypan blue, February 8, 11, 14, 17, 20, 24, 26, March 1 and 4, 1 c. c. each day.

March 5: Animal is stained a deep blue. Subcutaneous tissues are deep blue. There is a small area stained blue-black at the injection-point. Under the low-power a magnificent staining of all cells is seen. Deposits are deep blue and too small to be seen individually with the low-power except for scattered large, blue vacuoles evidently in macrophages. Cell types are distinguished only

by the occurrence of these mammoth blue vacuoles in small cells which are apparently macrophages, but which contain no greater actual number of dye deposits than the fibroblasts, if, indeed, they contain as many (fig. 76).

Under the oil the identification of macrophages is confirmed. They are rather small, rounded-up cells, varying in size and dye-content somewhat, but typically containing about a dozen very large vacuoles and also more numerous small vacuoles and a varying number of crystals free in the cytoplasm. The large vacuoles are deep blue and show all stages of crystallization of their dye-content; some are colorless, with blue crystals included. Small fat vacuoles are present also. Neutral red stains the vacuoles which are relatively free from the blue dye. In cells which are full of crystals the blue needles remain side by side with numbers of bright-red vacuoles. The crystals are then apparently free from vacuoles. Areas distant from the puncture-point and abdominal skin show a great preponderance of crystalline deposits in macrophages. The crystals are long and deeply stained and so numerous as to form "brush-heaps" in the cytoplasm. Janus green, 1:10,000 stains short mitochondrial rods and granules between the dye vacuoles.

Fibroblasts on the abdomen are filled with dye deposits which, while usually linear, are also vacuolar. The cells all over the body show a maximum reaction to the dye; syncytial masses are formed with two and three nuclei. The vacuoles when present are small and less deeply stained than the linear deposits, so that the deep-blue crystals and curved threads are the most striking part of the picture. Elongated triangles and comets are found also. The long threads, stained blue usually, but also colorless, are very numerous, especially in areas more distant from the puncture-point, and form amazing tangles in the cytoplasm. Neutral red, in films from the abdomen, stains the less deeply blue linear deposits and also the vacuoles, which are often almost as numerous as the crystals. In other areas where the deposits are all linear the parts of the crystals which are less deeply blue are stained red with the supravital dye. In these fibroblasts very few vacuoles reacting to neutral red are present, so that the cells appear almost wholly blue, even with neutral red. Janus green, 1:10,000, apparently brings out additional structures whose color and morphology are indistinguishable from the linear dye deposits.

That the crystals which we have observed in both types of cell do not represent the tingeing either of preformed or newly risen protoplasmic crystalline structures, such as occur naturally in some body-cells (e. g., *Sertoli cells*), *would appear to be quite conclusively proven by the fact that there is a specificity of the crystal form for each of the varying crystallizing vital dyestuffs employed.* We instance here the blue needles of trypan blue (figs. 74, 76), the long, pointed saffron needles of dye 245 (fig. 42), the amethyst prisms of dye 226 (figs. 77, 78, 79, 80), the rhomboidal plates of trisulfonazoblau (fig. 81), and the deep-red rods of T 148 (fig. 23).

4. The evidence furnished by "double-staining" experiments with two dyestuffs of the azo series, most beautiful when the dyes vary both in color and in the manner of deposition in the cell, is easier to follow in the thin, expanded fibroblasts than in the macrophages, and the data which we have presented under this heading for the macrophages are all duplicated by the performance of the fibroblast cells (figs. 44, 46, 49, 52, 53, 55, 56, 59, 61, 64).

The foregoing cytological evidence would now appear to amply justify the following statements:

1. The mitochondria of fibroblasts are not to any considerable extent "used up," even if they are in any way concerned in the phenomenon of the establishment of vital-dye deposits in the fiber-forming cells.

2. The "dye bodies" of fibroblasts represent actual accumulations in fluid, high colloidal, flocculated, or crystallized form of the actual dye substance used in the tests.

3. There is thus an identity in nature of at least the majority of the "vital structures" produced by azo dyes in the protoplasm of the fiber-forming and in the great phagocytic cells.

The marked difference already observed in the reactions toward azo dyes of these two great classes of connective-tissue cells constitutes, then, not an example of the reputed fact that actually different structures are tinged in these two cell categories, but of a fundamental difference in the biology of the two cells. It would seem appropriate, consequently, for us to rehearse very briefly the striking biological differences which make their imprint in the distinctive vital-staining effects which characterize the two cells.

CHARACTERISTIC DIFFERENCES IN THE MACROPHAGE AND FIBROBLAST RESPONSE TO AZO DYES.

We have already had occasion to remark on the success with which one may obtain by appropriate dosage deposits of the vital dyes only in the macrophage series of connective-tissue cells. We refer to the initial great engorgement of these phagocytes in the early stages of acute staining with high dosages of positive dyestuffs of the azo class and to the pure macrophage crystalline deposits which can be obtained by excessively dilute dosages with the crystallizable dyes. Both of these phenomena seem clearly referable to the increased power of absorption or more permeable character of the macrophage cells. The lesser permeability of the fibroblasts also accounts for the generally smaller deposits which are produced in these cells under all conditions of acute vital-dye dosage which are sufficient to affect both types of cell. It is indeed possible that it is the slower entry alone which produces not only smaller, but denser, concretion-like, or crystalline deposits in these cells, for we have seen that the pure-crystal picture can be produced in macrophages if only the rate of entry of dye into the same be very gradual, as is obtained by the lowest dosages which we have employed. And we have also seen that the use of dyes with a great diffusion speed (*e. g.*, dye T 148) succeeds in establishing quickly a typical vacuolar segregation-apparatus in fibroblast cells.

Though slower to respond to the vital dye, *the fibroblasts will, however, under long-continued dosage, invariably increase their dye-content and, with a chronic dosage, may come to resemble in their deposits so completely the macrophage cells that marked separation in the "dye bodies" in accordance with cell types no longer obtains* (figs. 82, 83).

Protocol: Rat 18–1, injected intraperitoneally with a 0.5 per cent solution of dye T 148, January 31 to February 25, 1. c. c. each day.

February 26: Animal is stained a bright crimson. Subcutaneous tissues are bright red. Under the low-power the dye-content of all cells is seen to be large and is of a deeper color than usual for even heavy stains with this dye, although here the vacuoles appear pink only, and in some cases a pale yellow-pink. Both elastic and white fibers seem more yellow in color than is normal. Cell types are not distinct under the low-power.

Macrophages can be distinguished under the oil by their dye deposits as well as by the general cell morphology. This distinction is not, however, striking, and types are often somewhat obscure without careful study of the individual cells. Dye vacuoles fill the cytoplasm of the macrophages. These vacuoles are uniform in size for a given cell and usually are about the size of mast-cell granules. Rare macrophages have vacuoles of considerable size. The color of the vacuoles is also variable. They are perhaps slightly deeper in shade than the fibroblasts, but some are very pale, almost without color. Small concretions are often present in the vacuoles. The most definite, points of differentiation between these and fibroblast vacuoles are their shape, which is always round (no linear structures—"thread bodies"—are present), and their uniformity in size (fig. 83). Neutral red 1:5,000 stains the vacuoles in macrophages electively a slightly deeper red than the vacuoles of fibroblasts. No linear structures are found in these cells. Janus green stains normal mitochondria.

Fibroblasts are loaded with dye vacuoles which have only a pale pink color. A few in each cell appear like fat vacuoles, but these are not numerous. The dye vacuoles are round, but more typically triangular, comet-form, rod-shaped, and bizarre shapes, apparently due to elongated vacuoles. They attain considerable size, but are on the average slightly smaller than the macrophage vacuoles. The linear forms are very common; many of them occur in each cell. They may even be more deeply stained than the vacuoles, though they are not bright red (fig. 82). Neutral red stains the dye vacuoles and emphasizes the linear forms. Janus green stains normal mitochondria.

(See also Protocol, Rat 18–2, p. 13.)

Attention may now be called to a most important physiological difference between fibroblast and macrophage cells. We refer to the reaction which these cells exhibit to supravital applications of weak neutral red (1:10,000). This basic dyestuff would appear to speedily enter both of these cell types with equal avidity, but it is never greatly concentrated in the segregation-apparatus of fibroblasts. This is true regardless of the fact that a large vacuolar segregation-apparatus may exist in the fibroblasts (*e. g.*, after vital staining with dye T 148). On the other hand, in a wholly characteristic way, the vacuoles of macrophage cells always concentrate weak yellow solutions of neutral red to a conspicuous color intensity (deep orange or red). We call this characteristic response of the macrophage the "neutral red reaction."[1] It is a fact that such weak solutions of neutral red electively tinge the vacuolar-apparatus of fibroblasts, even though there is a greater concentration of this dyestuff within the vacuoles of macrophages. As we have just remarked, in cases where the type of dye treatment and of dosage establishes a segregation-apparatus strikingly similar or identical in both macrophages and fibroblasts, the "neutral red reaction" applied supravitally to such vitally stained tissues immediately picks out with infallibility the macrophage cells. But if acid dyes may enter the fibroblasts only more slowly than the macrophages, they are also not free to leave these cells as easily as they do the phagocytes. This brings us to a consideration of decolorization.

Not the least interesting of the phenomena connected with the storage of dyestuffs is the subsequent liberation of these substances by the cell, so that, save for certain residual effects, a more or less perfect decolorization of the cells and tissues occurs. It is apparent from this fact that the concentration of these materials within the cytoplasmic confines of certain cells is a temporary, not a permanent, effect, and that the factors which operate to establish these deposits must continue in efficacy to insure their permanence. There is reason for believing that this behavior toward dyestuffs is merely a visible instance of the manner in which other materials, not immediately utilizable in the metabolism of the body, are conserved in the storage mechanism constituted by these cells. The discovery of what these substances may be is an attractive field for future investigation. Analogous phenomena are found in the discrete miscroscopic deposits of lipoids and glycogen which may occur when the body has to contend with an overabundance of these substances or their alimentary precursors, but the places of storage for these materials, and especially for glycogen, are differently located (*e. g.*, liver, striated muscle-cells, etc.),[2] and we have referred to this merely as an analogy,

[1] We have not been impressed with the advisability, as has Renaut, of separating protein-containing (rhagiocrine) from simpler liquid-containing (plasmocrine) vacuoles in the connective-tissue cells.

[2] One may also mention the accumulation of hemosiderin in the pancreatic parenchyma cells.

because "decolorization" or rather demobilization phenomena also result with these "metabolic" deposits. The formation or obliteration of such metabolic deposits can be seen to depend accurately on the specific content in them of the body fluids. Remarkable as it may seem, we have been for a long while in the possession of facts which show that a "balance" must exist between the intracellular deposits of some substances and the content of the fluids outside the cell, regardless of the fact that such materials are temporarily set apart from the general protoplasmic substance and would hence not seem free to participate in its exchanges.[1]

In the case of the dyes, the perfection of this "balance" between intra-cellular and extra-cellular dye concentration depends on the type of cellular deposits, for some dye granules easily shed their content into the blood-stream, while others are singularly resistant to such withdrawal. *Other things being equal, the intense local stains secured by the application of negative or highly colloidal dyes to the skin or peritoneal cavity are uniformly more permanent than stains produced by more diffusible dyes.* There are two possible explanations for this. We may be dealing more predominantly with coarse phagocytosis, or we may have to do with deposits produced in the usual manner (whatever that may be), but more concentrated and hence less soluble, or with deposits inherently less soluble than usual. While it could not be denied that in the immediate neighborhood of the injection the large particles of a negative dye could, and do, gain access to cells by coarse phagocytosis, the tissues further distant are also affected by these dyes. The intraperitoneal administration of a negative dye like oxamine blue may finally give us a very marked lymph-gland and bone-marrow stain. It is possible that the slight diffusible component of such negative dyes produces the staining of these distant tissues, but the same mechanism which permits large particles to enter lymphatic trunks from the peritoneal cavity may also permit direct entrance into the vascular systems, and the phenomenon which we have cited may be merely an elective filtering-out of these particles by true phagocytosis.[2]

[1] The metabolic deposits, however, do not, of course, occur, except under definite conditions of protoplasmic concentration or richness in these substances, and it is the protoplasmic content of the substance which in turn actually stands in "balance" with the content of the tissue juices or blood-stream. If the demobilization of metabolic deposits is brought about by enzyme action, as would seem, for instance, to be the case where glycogen in the liver cells is hydrolyzed by amylase to glucose, a prompt cessation of this hydrolysis occurs whenever the sugar-content of the liver cells accumulates through any cause which will prevent its prompt removal from the cells, and this would occur through changes in permeability of the cell membrane, as Bayliss (1915) suggests, or the fact that a high sugar-content of the body fluids prevented egress of the sugar. Croft Hill is cited as believing that the same enzyme in the latter circumstance may actually reverse its action and accomplish the synthesis of glycogen, which from its physical character belongs to the class of substances which can be concentrated in solid form in the cell.

[2] We have refrained from citing the staining of the lymph-glands which receive the lymphatic trunks draining the peritoneum, or the staining of the omentum or Kupfer cells of the liver, because all these instances are open to interpretation as "coarse phagocytosis," inasmuch as a peculiar biological mechanism permits coarser particulate matter (*e. g.*, India ink) to accumulate there than would gain wider distribution. It is certain that in cases where ultramicroscopic phagocytosis has been the only available agency to explain cell inclusions, the inclusions in question are more permanent and are not able to decrease their intracellular concentration except by a very slow process of liberation of constituent particles which escape the cell, *e. g.*, carbon or colloidal silver. To those who would look upon these last-mentioned deposits, however, as permanent "tatoos," it is only necessary to state that this also is a relative matter, *i. e.*, such deposits have the best insurance of permanency if they are concentrated in a particularly intense way at focal points in the tissue, such, for instance, as can be produced by actual subcutaneous puncture or as occurs in the adjacent lymph-glands which drain a particular area (bronchial lymph glandular anthracosis). Great caution must be exercised in the assumption of permanency for more generally distributed particulate matter. Lange (1909) has shown that the body is free from colloidal silver one month after intravenous treatment with itl "Das Gesammtergebniss unserer Untersuchungen kann sonach dahin zusammengefasst werden, dass im unmittelbaren Anschluss an die Einführung von Silber in Form von colloidalem Silber wohl eine allgemeine Vertheilung über den gesammten Organismus durch den Blutstrom stattfindet, das diese aber nur eine vorübergehende ist und dass, selbst von den Hauptablagerungsorten des Silbers, dieses in verhältnissmässig kurzer Zeit wieder zur Ausscheidung gelangt."

The lessened solubility of the deposits produced by high colloidal dyes is open to other explanations. Such lessened solubility may spring from the identical cause of lessened solubility of the crystalline dilute-dosage deposits which can be produced with some positive dyes, *i. e.*, they may have developed an extraordinary density merely because they are more slowly produced. This view would find some support in the undoubted slower diffusion powers of the colloidal dyes as well as in their lessened excretion. The diffusible part of the dye would consequently be actually slowly applied to these cells in very dilute dosage over a considerable period of time. This explanation, however, is negated by the relatively large and quickly formed deposits which may be produced, for instance, in the macrophages of the atretic follicles of the ovary when a high colloidal dye like afridol blue is administered by the peritoneal route. The deposits are large in size and quickly produced; indeed, an extensive staining of the subcutaneous tissue shows that fairly rapid diffusion has ensued. The deposits are also remarkably permanent. It is hence certain that the higher colloidal dyes tend to make less soluble accumulations within the cell. This would appear to be an inherent physical property of the compounds in question comparable to their sensitivity to electrolytes, absorptive power, etc., and it is a necessary field for future investigation.

In addition to these facts of the greater permanency of colloidal dye deposits it is necessary to refer to the fact that a similar permanency also rarely characterizes the deposits of certain very diffusible brilliant vital dyes of the azo series, substances which also establish a large vacuolar segregation-apparatus. In these instances the dye deposits by no means remain in their pristine form and extent, for much of the dye substance disappears on decolorization. A residuum of the dye substance, however, would appear not to be able to escape the vacuolar-apparatus where it undergoes a unique and interesting concentration to small concrements. A great number of larger vacuoles are thus replaced by smaller, intense amorphous masses. This peculiarity is exemplified by the behavior of vital new red (figs. 84, 85).

Protocol: Rat 312, injected intraperitoneally with a 1 per cent solution of vital new red, 1917, April 28, May 1, 7, 15, 24, 29, June 5, 9, 12, and 16, 0.5 c. c. each day; June 20 and 23, 4 c. c. each day.

1918, March 20: Animal is stained a bright pink. Skin of thigh light pink. Under the low-power, cell types can not be made out with certainty, but it is seen that a considerable number of dense-red deposits occur in the great majority of the cells.

Under the oil it is readily seen that the main mass of the dye is in the fibroblasts, where it occurs in rather uniform-sized, deep-red, angular deposits. These vary in number from perhaps 15 or 20 to 75 or 80. Neutral red does not disclose any other bodies, or at most very few other bodies, of the vacuolar type (fig. 84). It is evident that such deposits constitute a lifetime pigmentation of these cells.

The macrophages are distinguished by a sparser number of vital-dye deposits and by the smaller size of these as a rule, though instances of large deposits in macrophages can be found. Neutral red shows what is probably a normal vacuolar system in these cells. The reaction gives at most a pale orange-color to these bodies and convinces us that they have lost their content of the vital dye (fig. 85).

While there thus occur specific differences in the deposits produced by various dyes, it is also true that the manner of establishment of the dye granules in the

case of any one dye will influence greatly their rate and extent of decolorization.[1] Through our studies we now know that just as permanent dye effects may be secured from some of the less colloidal, rapid-staining positive benzidine dyes as from pyroll blue or any of the negative dyes, for we have merely to use frequent but very low dosage to attain this result. *The cellular deposits produced by much slower, very dilute dosage enjoy a very much longer "post-experimental" life.* The dye in them is obviously less able to leave them and stream through the cell's protoplasm to reach the extracellular juice. Or if decolorization be due also to chemical destruction of dye depots, such denser depots withstand longer attack on the part of the cell. It is unlikely that any part of the decolorization process consists in the mechanical expulsion or separation of the dye from its vacuoles or of the dye vacuole in entirety from the cell, such as we observe in the shedding of food vacuoles in certain protozoa; for were this the case it would be difficult to see how differences in the state of the dye within the vacuole could produce the observed differences in the rapidity of the decolorizing process. There are, in fact, no other evidences of such a purely mechanical expulsion of the dye deposits as constituting the decolorizing act. This material is never encountered just without the confines of the cell. Indeed, other evidence convinces us that the dye escapes by diffusion from the places of its detention within the cell. In the case of those dyes which, like T 148, produce large segregation vacuoles in the fibroblastic protoplasm, the decolorizing act leaves these cells practically free from dye but with the full number of the dye-inclusion vacuoles filling the protoplasm. The decolorization phenomenon thus furnishes one of the strongest reasons for believing that vital-staining effects are produced by the cell's acceptance of substances which possess power of diffusion; for this would appear to be the best available explanation of the method of egress of the dye from its intracellular depots. If the dye does leave its place of agmination inside the cell by such processes, there is furnished all the necessary proof that it could originally enter the cell by such means, and there exist no grounds for the hypothesis that the azo dyestuffs can enter these cells only by virtue of a truly phagocytic act.

Nowhere does the contrast in behavior between macrophages and fibroblasts show itself more emphatically than in the decolorization phenomena. The connective-tissue producing cells not only show relatively less decolorization, but, if one will wait long enough, will be found to have absolutely more dye in them than have their

[1] It is of interest to note that Goldmann laid great weight on the necessity of a gradual rather than a rapid attainment of a vital stain. He called particular attention to the great permanency characterizing staining by the slower method, and felt that more normal conditions were secured by slow application, such as by the cutaneous method with pyroll blue. Speaking of the "toxic" effect of dyes which, like trypan blue, tinged the whole animal speedily and intensely, Goldmann's impression has some basis in fact. It is possible to produce tissue new-growths merely by the application of larger doses of the benzidine dyes (endothelial giant cells in the sinusoids of the liver, bone-marrow, and spleen), but dyes which stain more quickly are not necessarily more toxic. Ease of decolorization, however, follows strictly ease of staining, and those vital stains which are produced with speed with benzidine dyes fade with corresponding celerity. Decolorization need not be allowed to occur, however, if one will only plan his dye dosage with appropriate relation to the time at which tissues are to be examined for vital-staining effects; and the greater speed of staining produced by the benzidines makes them indispensable for some studies in which a slower method would not yield results. Goldmann's unfavorable opinion fortunately did not induce Schulemann (1912) to desist from his valuable studies on trypan blue, in which for the first time were shown all the pyroll-blue cell pictures, produced with greater ease, even if with less permanency, by intravenous dosage. It was also quickly apparent to experimental workers like Gross, Wieszeniewski, MacCurdy, Evans, Winternitz and Bowman, and many others, that in tissue changes of rapid history the application of the trypan class of dyes rather than the slowly acting dyes would be necessary in order to test the staining possibilities of altered or of new tissue components from time to time.

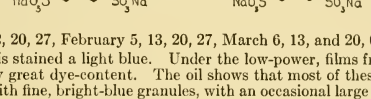
macrophage sister cells, initially gorged with many times the fibroblast quantity. Nothing is perhaps more surprising that the exploration of the skin of an animal which has once been profoundly stained in the typical "acute" dosage and is then permitted to live to a post-experimental period of a half year or more. The macrophage deposits, originally overwhelmingly more abundant, larger, and more brilliant than those of the fibroblasts, have, except in some paravascular cells, now almost entirely disappeared, even though colorless vacuoles indicate occasionally their former extent. The fibroblasts, on the other hand, may maintain their former dye-content unweakened in brilliancy and are consequently not only the cells now carrying most of the vital dye, but may even seem to be the only cells which possess the latter. We were first acquainted with these facts, to our great surprise, by the examination of the skin of animals which had been permitted to live for relatively long periods after the end of treatment with vital dye.

Protocol: Rat BII 955, injected intraperitoneally with a 0.5 per cent solution of vital new red, 1916, July 9, 10 c. c.; July 23, 29, and August 6, 7 c. c. each day.

1917, March 1: Light red color shows at base of ears. Subcutaneous tissue generally deep pink. Films from the thigh show no differentiation of cell types under the low-power, except where deep-red macrophages outline the capillary walls. The oil shows very fine, dotlike, deep-red granules and colorless vacuoles in macrophages. The paravascular macrophages are filled with round, red vacuoles, but at times show a fading out of some vacuoles, with formation of the small, deep-red granules. Fibroblasts contain medium-sized, round and angular, deep-red deposits. There are smaller granules also in fibroblasts, and some, indeed, have no brilliant, large deposits; great numbers of these cells, however, contain red dye deposits approximately the size of those in the macrophages along blood-vessels.

In films from the abdomen and side more of the macrophages with large red vacuoles are present out in the tissue away from the blood-vessels. Janus green shows normal mitochondria in both types of cell.

Protocol: Mouse 57, injected subcutaneously with a 0.5 per cent solution of afridol blue (a deep bluish-purple colloidal dye made by combining 1 molecule dichlorbenzidine with 2 molecules of the H acid, and hence with the formula



January 5, 12, 20, 27, February 5, 13, 20, 27, March 6, 13, and 20, 0.2 c. c. each day.

June 18: Animal is stained a light blue. Under the low-power, films from the abdomen show many cells with a very great dye-content. The oil shows that most of these are fibroblasts whose cytoplasm is packed with fine, bright-blue granules, with an occasional large vacuole; these deposits extend to the limits of the cell's processes, leaving only a narrow zone of cytoplasm (usually next the nucleus) comparatively free from dye. Macrophages escape notice at first on account of their relatively small dye-content. The dye is present in the form of vacuoles varying from an occasional one larger than the nucleus to minute deposits which are possibly concretions within colorless vacuoles.

Tissue at area of injection is deep blue. Fibroblasts here are packed with dye. The color of the small fibroblast deposits varies from a purple to a distinctly green blue indistinguishable from janus green. Except for a few green-blue "threads" and rods in fibroblasts at the area of injection, all deposits are vacuolar or round. "Threads" at the injection-site are rare and not striking. They are connected with the vacuolar dye deposits. Macrophages here are more heavily laden than over the abdomen and their vacuoles are deep blue. They contain some fat droplets. Dye deposits are so numerous here as to make study of individual granules difficult, but one is impressed with the similarity of them in all parts studied.

Protocol: Mouse 54–8, injected intraperitoneally with a 1 per cent solution of trypan blue January 5 and 20, 0.5 c. c. each day; January 27, February 5, 10, 13, 17, 20, 24, 27, March 3, 6, 10, 13, 17, 20, 23, 27, and April 10, 0.25 c. c. each day.

June 21: Animal is bright blue. Cells are full of dye visible with the low-power, but types are not clear. Many cells with large deposits seen with this power under the oil prove to be fibroblasts and not macrophages. The two types are distinct on the whole.

Macrophages are neither numerous nor striking and are easily overlooked. Their dye-content varies from one or two small concretions in colorless vacuoles to several large, deep-blue vacuoles and smaller deposits. They are rarely as deeply stained as the fibroblasts.

Fibroblasts are well packed with regular, bright-blue granules, usually somewhat triangular or linear, though often round and square. The size of these varies slightly in different cells, some having so large vacuoles as to be identified as macrophages at first. Many possess also a single mammoth deep-blue vacuole similar to those in macrophages.

Dye deposits are, then, invariably more permanent in the fibroblasts than in the macrophages, and time and again, after acute dye dosage which pigments both types of cell, a period of freedom from dye treatment gives us at length areolar tissue in which only the fibroblasts have retained their stain, while the macrophages have lost their initially great content, due to the speedier decolorization undergone by these cells.

In an animal handled in this way, but now injected with a single high dose of a positive azo dye, different in color from that first used, the macrophages of the areolar tissue of the entire body exhibit their customary acute reaction and are deeply tinged with the last vital color employed. If we examine the tissue quickly during the interval preceding any appreciable dye deposition in the fibroblasts, we may observe differential color-staining of the two cell types, the fibroblasts carrying the earlier dye alone, the macrophages the dye last employed.

Protocol: Mouse 58, injected subcutaneously with a 0.5 per cent solution of trypan blue, July 27, 31, August 3 and 8, 0.5 c. c. each day; September 14, 21, 27, October 3, and 8, 5 min. each day; October 16, 10 min.; October 20, 4 min. Injected intraperitoneally with a 2 per cent solution of dye T 148, December 1, 0.2 c. c.

December 5: Low-power shows numerous cells filled largely with red (some blue) dye, contrasting with fibroblastic cells containing blue deposits.

Under the oil, though it is somewhat difficult to identify all the red cells, the majority are seen to be macrophages. These may, however, show large blue vacuoles or a blue tinge in red vacuoles.

Fibroblasts are usually closely packed with blue deposits, round and sharply linear in form. It is true that on closer scrutiny some show pink threads and small pink vacuoles. In these instances the pink threads are connected with blue dye deposits. Janus green shows filiform mitochondria.

It is remarkable that the majority of fibroblasts have not been affected by the red dye; also that the majority of macrophages have no blue dye.

It is interesting that this differential fading, or decolorization, which gives us an actual subsequent predominance of the fibroblast stain, is also shown in the case of vital-dye substances which from their colloidal nature decolorize slowly. In other words, the differential behavior of the two types of cell is still evident, though a longer period of post-experimental life is necessary before much decolorization can take place. This is the case, for instance, in animals which are treated over a time interval of about 75 days with afridol blue and allowed to decolorize during a period of 90 days. (See Protocol, Mouse 57, page 41.)

We have reserved for final consideration a further discussion of the peculiar tendency of the segregation-apparatus of fibroblasts to assume a linear form. It is

this tendency, doubtless, which has been mainly responsible for the confusion of the fibroblastic segregation-apparatus, often filiform in character, with the mitochondria. In the case of the fibroblasts it is in fact always possible to trace every transition between small, globular vacuoles or granules and elongation of them into bacillus-like, rod-like, or filiform structures. These forms must not be confused with the crystallization process, which also gives delicate rod bodies and which can occur equally well in the macrophage cells. A filar form of the segregation-apparatus in macrophages, however, never occurs. This is hence a distinctive reaction displayed by the fibroblastic cells. The filar structures may under certain circumstances attain an altogether remarkable exaggeration, producing complex entanglements of such "threads." These might legitimately be taken as hypertrophied mitochondria were criteria for distinguishing them not at hand (fig. 86).

Before discussing the affinities of these structures, it would be well for us to comment on the extent and characteristics of such "thread bodies" under various experimental conditions. They are never displayed by the fibroblastic protoplasm of the mouse to the same extent as in the rat; and in the latter animal they are typically produced only by certain dye substances and in particular areas. The "thread bodies" are typically produced by trypan blue and its biological homologues (figs. 11 and 87). *While the dyes which greatly enlarge the segregation-apparatus of the fibroblasts (dyes 11, 228, and T 148) do not usually produce filar modifications of the same, they nevertheless do accomplish this to some slight degree* (fig. 82), and one may see among the usual vesicular structures pointed prolongations of them producing comet or comma forms, or, again, rather sturdy long "threads." *Even these dye substances, however, produce extensive "thread bodies" in areas where they have opportunity to affect the fibroblasts in a particularly intense way, e. g.,* in the areolar tissue next the tunica vaginalis of the scrotal sac. Here the fibroblastic segregation-apparatus exhibits its maximum tendency to undergo filar modifications and great, rounded-up, true fibroblastic cells, whose protoplasm is full of a confused tangle of "threads" may result (figs. 7 and 9). We have never under any circumstances seen these structures in the protoplasm of the macrophage cells, nor do they in our experience ever characterize any other cells of the body (*e. g.,* mesothelium, endothelium, lymphocytes). We have come to regard them, consequently, as distinctive characteristics of the fibroblasts, though they are not invariably displayed by the latter. The filar structures carry weak but readily appreciable amounts of the vital azo stains, which, since they can be demonstrated to develop from the vesicles of the segregation-apparatus, must be derived from that source. They react like the segregation-apparatus to supravital tinction with neutral red and, indeed, a considerable abundance of them, on account of their very weak color, may be overlooked unless neutral red is applied to the cell. They are particularly prone to develop with trypan blue and its biological homologues. Vacuolar enlargements may occur at either end of them, or along their course. Their delicacy and straightness may sometimes lead one to interpret them as crystalline products; but that they are not crystals of the dyes which produce them can be shown conclusively by the employment of dyes whose crystals possess a somewhat different

color from their solution color, whether in the test-tube, "thread bodies," or vacuolar-apparatus of cells (fig. 77). The filar structures constitute much more faithful copies of filiform mitochondria than of vacuoles, and the feasibility of separating them from the mitochondria must now be entered into. We may rehearse some of their characteristics which have induced us to take the view we have presented.

1. They are actually connected with part of the segregation-apparatus and in early stages of their formation transition stages between typical vesicles of the segregation-apparatus and these filar structures can be seen.

2. In common with the segregation-apparatus, they stain electively with neutral red.

3. They are not stained electively by any of the mitochondrial methods.

4. They do not undergo morphological alterations post-mortem, even after many hours' standing.

The intensity of the vital color in the filar bodies is never very great. This is all the more remarkable since they are most abundant where conditions of severe dosage of the cell have ensued, and it may hence be thought that they represent an unusual stimulation effect of these cells and that they possibly stand in some obscure relation to the fiber-forming proclivities of the same elements. That they are not collagenous fibers is evident by their failure to stain by any of the tinctorial methods for these structures, even though none of such methods is highly elective, for by all methods which deeply stain the extracellular collagenous fibrils in their immediate neighborhood these intracellular "threads" remain untinged. That they may be peculiar degeneration products, resulting from protoplasmic injury due to the direct effects of the stain, is suggested by the discovery of Bachmann (1912) that soaps, for instance, may be precipitated in this peculiar filar form. The structures do not, however, give any of the reactions of soaps or lipoids, and we are unfortunately still without a satisfactory clue as to their significance. The outstanding fact of importance to our argument, however, has been determined—their relation to the segregation-apparatus, not to the mitochondrial system of the fibroblastic cells.

It is appropriate, lastly, for us to refer to the light which these investigations shed on the question of cell specificity in the connective tissues, especially in view of the fact that where this question has been most carefully studied—in the histology of aseptic inflammation, wound-healing, and tissue cultures—various hypotheses and supposed facts of cell transformation have been advanced. Nothing which we have seen leads us to the view that these fundamental cell types are not constantly, even under the most diverse conditions, maintained.[1] Though we have described great divergencies in the type of staining displayed by both the macro-

[1] Such a statement is indeed quite different from that which concerns the lymphocytic origin of the macrophage or polyblastic cells and for which evidence has been brought forward by many of the special students in this field. We have not given the latter matter our attention, but studies carried out in this laboratory on the mononuclear blood-cells indicate that a segregation-apparatus can be clearly demonstrated in these cells, though there is general recognition that their ingestion of azo dyestuffs is forbidden as long as they are in the blood or lymph stream.

phage and fibroblast cells, we have experienced few instances where vital dyestuffs have not displayed with peculiar force the differing physiology of these cells. Maximow, indeed, in his last studies, which were with tissue cultures (1916), was able to still identify as fibroblasts the greatly changed, vacuole-filled elements which in old cultures constitute these cells, and his figures are not unlike those which may be found with those vital stains which, we have discovered, easily affect fibroblasts in this way (*e. g.*, T 148).

This is not a transformation of the whole physiology of these cells. It will be remembered that, under conditions of inflammation, Renaut has seen such striking changes in the fibroblasts as to lead him to declare the assumption of a "rhagiocrine" character on the part of these cells. But it is difficult for us to be certain as to what Renaut would have us infer from such statements. It would appear evident, however, from his account of the development of the connective tissues, that he considers the appearance of a segregation apparatus in fibroblasts in places of inflammation as a reversion to a primitive trait on the part of fibroblastic cells. But we would emphasize, as he does not, that the fibroblasts by this act do not become macrophage cells. We have already shown that a small vacuolar segregation-apparatus normally exists and can be greatly hypertrophied in the fibroblast. Kiyono, who sought to reject our theory of the ingestion of dye on the part of fibroblasts, argued that no instances of the inclusion of foreign material by these cells have been encountered, but they ingest metallic sols, India ink, and even larger particulate matter, so that the act which we term phagocytosis takes place, though admittedly to a lesser degree, in the case of these cells.

Renaut has also called attention to what he believes to be the more outspoken "rhagiocrine" function of young fibroblasts when compared with adult cells. Indeed, his whole description of the development of the connective-tissue cells indicates that he believes that the ranks of the fibroblasts are continually being augmented by the process of a wandering-in and fixation of macrophage cells which gradually change their character, so that in young animals there are many "rhagiocrine fibroblasts," in old ones very few, throughout the areolar tissue of the body.

While it might be deemed that our whole study has accentuated in an unmistakable manner the similarity of reaction of macrophage and fibroblast cells, distinctive differences in this reaction to vital dyes are never obliterated. Fibroblasts are with some dyes and dosages affected in an identical way as are the macrophages in other cases, but not identically with their own macrophage sister-cells. Our study of the areolar tissues of newborn rats demonstrates that at this time the two distinct cell types are already present and that they behave specifically toward vital and supravital stains. This statement may be made impregnable by limiting it to the affirmation that type segregation is at least true for the overwhelming majority of the connective-tissue cells.

In the beginning of studies on the living areolar tissue one will repeatedly descry what he believes to be an intermediate cell type and which resembles some of the figures of Renaut. These are best characterized by the statement that certain ex-

panded cells, similar in many respects to the fibroblasts with which they may anastamose, possess a more considerable segregation-apparatus and deeper-staining nuclei with neutral red than do the majority of the fiber-forming cells. These cells are almost invariably at the periphery of the tissue, never in its depths; they appear to us instances of the too concentrated action of the supravital dye (neutral red), the vacuole-producing proclivities of which are not inconsiderable. Certain it is, that as one proceeds in his studies the detection of intermediate or unclassified cells occurs with less and less frequency.

When we enter the realm of the pathological transformation of these cells, the problem gains in difficulty and complexity. We have never seen instances of the undoubted production of macrophages from fibroblast cells, though the latter may have installed in them an elaborate vacuolar-apparatus through prolonged treatment with positive azo stains or, in particular positions, display such an apparatus even in the case of negative dyes. It would appear that our experiments with large quantities of high-colloidal and not easily absorbed dyestuffs reproduce adequately the phenomenon of aseptic inflammation, but such experiments have failed to yield us instances of any considerable true transformation of fibroblasts into macrophage cells.

We are assuredly already acquainted with enough facts in the histogenesis of the cellular elements in connective tissue to recognize a fundamental genetic relationship even of most diverse elements, such as endothelium and leucocytes, as well as the types of connective-tissue cells. The endothelial production of intravascular macrophages has been studied by Evans, Bowman, and Winternitz and has since been confirmed by Kiyono, Aschoff, and others by the application of the method of vital stains. It has long been noted by the pathologists—before elective methods for proof were in their hands (Mallory); and we have recently had too many reiterations of an actual origin of the polyblasts in inflammation from lymphocytes to deny the possibility of macrophage production from the mononuclear blood-cells.

Our ideas of the specificity of cell types must indeed, as is apparent from the researches of Harvey from Bensley's laboratory, take up the question as to how far the assumption of a specific cell function or structure makes either the continuance of this or cell death the inevitable result when new surroundings confront the cell (*e. g.*, nerve and myocardial cells). Harvey (1906) showed that the chief cells of the gastric glands in the region of a new artificial pylorus lost their distinctive characteristics and reverted to the type of muciparous cells. Our conception of specificity among the elements here specially studied would hence take its best formulation in the belief that the fibroblast does in general represent more nearly an irrevocable specialization of the cell, but that this is not the case with the great phagocytic elements—the macrophage cells. This is in harmony with the recent findings of Maximow that the culture of free peritoneal macrophages will undergo differentiation and yield colonies of fibroblasts, but a change in the reverse direction, *i. e.*, from fibroblasts to wandering elements, has not been described. A remarkable vitality and regenerative power must nevertheless be ascribed to the

fibroblasts, a fact familiar to us in the production of granulation tissue and evidenced in the most spectacular way by their exuberant growth in tissue cultures.

This is not the place for a discussion of the interesting question of the affinities of either of the areolar cell types with mesothelium, but the single statement may be made that azo dyestuffs have very distinctly helped our power of discrimination and separation of these cells. In the specificity of this cell type, which is championed by Tschaschin against the views of Ranvier, Schott, and Weidenreich, we find ourselves in hearty agreement. The normal mesothelium is even freer from vacuolar bodies than are the fibroblasts and hence has closer affinities with the fibroblasts than with the macrophage cells.[1] Its cells can always be distinguished from both of the connective-tissue elements by means of the vital azo dyes. Such a distinction, it is again to be emphasized, is not made on the basis of a unique reaction to the dye different in kind from that displayed by the connective-tissue cells, but by the fact that with any particular dosage and dye the form, size, distribution, and abundance of the mesothelial segregation-apparatus thus called into existence to house the vital dye will assume characteristics no less peculiar for the mesothelial than for either of the connective-tissue cells. Mesothelial cell deposits are usually minute ones. Even with protracted intraperitoneal dosage, they do not, in our experience, show the filar structures occasionally provoked in the fibroblasts, nor do they acquire a vacuolar apparatus as great as that possessed by the subperitoneal contiguous macrophage cells, even in cases where they are completely filled by dye storage, as is frequently true of the mesothelium of the spleen.

The mesothelial cells can also become the seat of true crystalline deposits of the vital dye either as a result of great dosage (our saturation crystals) or by the method of prolonged low dosage with some of the crystallizable members of this extensive series of dyes. Claims for the participation of mesothelium in true fibroblast production would not, from any theoretical deductions, be wisely denied, but this will have to be investigated with the aid of specific cytological criteria furnished by an agent or agents at least as satisfactory as the vital azo dyes. The methods hitherto employed have not been adequate for such a task. As far as the occurrence of macrophage production from the mesothelium is concerned, it will be recalled that this has indeed been taken by many observers as the source of the free cells of the peritoneal cavity. A renewed study of this question with better methods of discrimination would likewise be indicated, and more especially since so good an authority as Weidenreich has been willing to derive the macrophages of the peritoneal exudate from almost every possible source in the mesothelial, the wandering, the hemotogenous, or the connective-tissue cells. If the claims for the participation of the mesothelium in macrophage or fibroblast production are, then, perhaps not justly stigmatized as groundless, it yet appears certain from studies with the benzidine dyes that mesothelium itself is produced only from mesothelium.

[1] Renaut has also been struck with the absence of a "rhagiocrine function" in the normal definitive pleuro-peritoneal endothelial cells. These elements, however, receive azo-dye deposits which are not less characteristic than those of the other vitally stainable cells.

Tschaschin's claim, in his experimental study of peritonitis, for the feasibility of identifying mesothelium by means of the vital stain, finds the fullest corroboration in our experiments. We can not help but feel that decided support is thereby given to the belief that the mesothelial elements constitute a distinct cell strain *sui generis* and in the case of injury must repair themselves.

SUMMARY.

1. The two great cell strains of the connective tissue of mammals—the fibroblasts and the macrophages—exhibit a pronounced and characteristic difference in their reaction to intra-vitam acid dyes.

2. This difference shows itself in the appearance of two sharply separated types of response to the dye—in the size, form, and number of the vital dye "granules" and in the fact that these types of vital-dye response are associated with those other characteristics which permit us to designate fibroblast and macrophage cells.

3. The mitochondrial apparatus of the connective-tissue cells can not be said to be electively stained by means of the vital acid dyes.

4. The vital-dye "granules" in the case of both fibroblast and macrophage cells are neither chemical combinations of the dye with the protoplasm nor physical tingeing of pre-existing cell-organs, but are actual accumulations within the cell of the vital dyestuff in fluid, high-colloidal, flocculated, or crystalline form, in accordance with the conceptions of Evans and Schulemann.

5. The ingestion of these dyestuffs is usually associated with their separation from the living protoplasm by virtue of a segregating power of the cell; for such segregation, granules, minute vesicles, and vacuoles of many sizes may be created in addition to those already present in the cell. We have called the ensemble of these structures the "segregation-apparatus" of the cell.

6. The power to store vital dyestuffs is, on the part of the macrophage, greatly in excess of a similar capacity shown by the fibroblast cell.

7. The macrophage vital-dye deposits are, conversely, more susceptible of decolorization and less permanent than are the more minute deposits in the fibroblast cell.

8. The brilliant vital stains which may be secured with the acid dyes bring to view in an unmistakable way an underlying physiological difference in the macrophage and fibroblast cells, and in particular display their differing capacity to ingest, segregate, and store substances of the same chemical or physical state as characterize solutions of the dyestuffs belonging to the acid-azo class.

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EXPLANATIONS OF PLATES.

The figures represent in each instance actual laboratory drawings of the living cell. The more fugitive structures (*e. g.*, mitochondria) were sketched first, the more substantial ones later. In all cases the drawing was carried through to completion in the presence of the material itself. Proportions have been maintained with the camera lucida. Where not otherwise stated, the magnification is 2,000 diameters; critical illumination with white light, and a Zeiss apochromatic 1.5 mm. oil-immersion lens and ×8 compensating ocular were used. The position of the nucleus is indicated, but the drawings do not aim to convey information relative to the character or boundaries of the protoplasm, except as these may be indicated by the deposits of vital dye.

FIG. 1. Subcutaneous tissue from thigh of rat which has received six 2 c. c. intraperitoneal doses of 0.5 per cent trypan blue over a period of 6 days. Examined 1 day after last injection. (See protocol Rat 10, p. 7.) Physiological saline. ×160.

FIG. 2. Fibroblast and macrophage. Subcutaneous tissue from thigh of rat which has received a daily 2 c. c. intraperitoneal dose of 0.5 per cent trypan blue for 4 days. Examined 2 days after the last injection. (See protocol Rat 333, p. 8.) Physiological saline.

FIG. 3. Macrophage. Subcutaneous tissue from thigh of rat which has received four 1 c. c. intraperitoneal doses of 1 per cent trypan blue over a period of 10 days. Examined 2 days after last injection. (See protocol Rat 31–2, p. 7.) Physiological saline.

FIG. 4. Fibroblast from same case. Neutral Red 1:10,000.

FIG. 5. Fibroblast. Subcutaneous tissue from thigh of rat which has received six 2 c. c. intraperitoneal doses over a period of 17 days of a bright red, highly diffusible dye (dye H 11) produced by the combination of 1 molecule dichlorbenzidine with 2 molecules beta naphthylamine 3.6 disulphonic acid. Examined 3 days after the last injection. Janus green 1:10,000. The dye deposits are pale pink, the mitochondria bright blue-green.

FIG. 6. Macrophage from same case. Neutral red 1:10,000.

FIG. 7. Fibroblast. Subcutaneous tissue from serotum of rat which has received 20 daily 1 c. c. intraperitoneal doses of dye T 1835, the purple dyestuff made by the combination in acid of 1 molecule o-tolidine with 2 molecules 1.8 amidonaphthol 3.5 disulphonic acid. Examined 20 days after last injection. Neutral red 1:10,000. Note the extensive formation of "thread bodies" from the segregation-apparatus.

FIG. 8. Macrophage from same case. Neutral red 1:10,000.

FIG. 9. Fibroblast. Subcutaneous tissue from serotum of rat which has received fourteen 2 c. c. intraperitoneal doses followed by four 4 c. c. doses over a period of 53 days with the blue negative dyestuff (dye H 7), made by the combination of 1 molecule dianisidine with 2 molecules alpha naphthol 4 monosulphonic acid. Examined 70 days after last injection. Physiological saline. Globular vacuoles, three of notable size, and great numbers of slender threads.

FIG. 10. Macrophage from same case. Physiological saline.

FIG. 11. Fibroblast. Subcutaneous tissue from thigh of rat which has received two 2 c. c. intraperitoneal doses, followed by two 1 c. c. doses of 1 per cent trypan blue over a period of 10 days. Examined 36 hours after last injection. Physiological saline.

FIG. 12. Macrophage. Subcutaneous tissue of normal rat. Neutral red 1:5,000.

FIG. 13. Fibroblast. Subcutaneous tissue of normal rat showing the very slight segregation-apparatus characteristic of these cells. The number of these is even somewhat in excess of what is typical. Neutral red 1:5,000.

FIG. 14. Mitochondria of macrophage from subcutaneous tissue of normal mouse. Janus green 1:10,000.

FIG. 15. Macrophage. Subcutaneous tissue from abdomen of rat which has received three 4 c. c. intraperitoneal doses of 0.5 per cent of the bright-red vital dye T 148 (a combination of o-tolidine with 2 molecules of alpha naphthylamine 4.8 disulphonic acid) over a period of 11 days. Examined 3 days after last injection. Janus green 1:10,000. The vital-dye granules are light pink, the mitochondria greenish blue.

FIG. 16. Fibroblast from same case. Janus green 1:10,000.

FIG. 17. Macrophage. Subcutaneous tissue from thigh of rat which has received 10 daily 1 c. c. intraperitoneal doses of a 0.5 per cent solution of the deep brownish-red vital-dye 229 produced by the combination of benzidine metadisulphonic acid with 2 molecules beta naphthylamine 7 monosulphonic acid. Examined 3 days after last injection. Janus green 1:10,000. The dye vacuoles are brownish red, the mitochondria blue green.

FIG. 18. Fibroblast from same case. Neutral red 1:10,000.

FIG. 19. Mitochondria of a macrophage. Subcutaneous tissue from abdomen of rat which has received 4 c. c. intraperitoneal doses of 0.5 per cent dye T 148 over a period of 16 days, injected every fifth day. Examined 13 days after last injection. Janus green 1:10,000. The dye vacuoles, which were extremely abundant, are not outlined.

FIG. 20. Macrophage. Subcutaneous tissue from thigh of rat which has received six daily 2 c. c. intraperitoneal doses of 1 per cent T 148. Examined 1 day after last injection. Neutral red 1:10,000. This was one of the most completely engorged macrophages found, though all have one or more vacuoles of great size.

FIG. 21. Fibroblast from same case. Neutral red 1:10,000.

FIG. 22. Macrophage. Subcutaneous tissue from thigh of rat which has received 18 daily 1 c. c. intraperitoneal doses of 0.5 per cent T 148. Examined 1 day after last injection. (See protocol Rat 18-2, p. 13.) Neutral red 1:10,000.

FIG. 23. Fibroblast. Subcutaneous tissue from abdomen of same case. Neutral Red 1:10,000. The peculiar deep-red, rodlike crystals of the vital dye are apparent, part of them vacuolar in origin, part intervacuolar.

FIG. 24. Macrophage. Subcutaneous tissue from thigh of rat which has received 7 daily 4.5 c. c. intraperitoneal doses of 1 per cent Baumwollrubin (B.A.S.F.). Examined 4 days after last injection. (See protocol Rat 34, p. 13.) Neutral red 1:10,000. It is surprising that the macrophage vacuoles are no larger than those characterizing the fibroblasts.

FIG. 25. Fibroblast. Subcutaneous tissue from thigh of same case. Janus green 1:10,000. Excellent contrast of the bright blue-green mitochondria with the pink segregation-apparatus, which in some few cases shows filar modifications.

FIG. 26. Macrophage. Subcutaneous tissue from thigh of rat which has received 30 daily 1 c. c. intraperitoneal doses of 2 per cent New Bordeaux L (B.A.S.F.). Examined 36 hours after last injection. (See protocol Rat 47, p. 14.) Neutral red 1:10,000.

FIG. 27. Fibroblast from same case. Neutral red 1:10,000. No marked difference in the size of macrophage and fibroblast vacuoles is apparent.

FIG. 28. Macrophage. Subcutaneous tissue from thigh of rat which has received 10 daily 1 c. c. intraperitoneal doses of 0.5 per cent dye 230, a red diffusible dye made by combining benzidine o-disulphonic acid with 2 molecules of beta naphthylamine 7 monosulphonic acid. Examined 3 days after last injection. (See protocol Rat 94, p. 14.) Neutral red 1:10,000.

FIG. 29. Fibroblast from same case. Physiological saline. No marked difference in the size of macrophage and fibroblast vacuoles is apparent.

FIG. 30. Macrophage. Subcutaneous tissue from thigh of rat which has received sixteen 1 c. c. intraperitoneal doses of 1 per cent 1212 *d*, a very diffusible bright-red dye made by combining 1 molecule o-tolidine with 2 molecules of beta naphthol 8 monosulphonic acid, over a period of 19 days. Examined 1 day after last injection. (See protocol Rat 19, p. 15.) Neutral red 1:10,000.

FIG. 31. Fibroblast. Subcutaneous tissue from thigh of same case. Neutral red 1:10,000. No marked difference in the size of macrophage and fibroblast vacuoles is apparent.

FIG. 32. Macrophage. Subcutaneous tissue from thigh of rat which has received fourteen 2 c. c. intraperitoneal doses, followed by four 4 c. c. doses of 0.5 per cent dye H 7, made by combining 1 molecule dianisidine with 2 molecules alpha naphthol monosulphonic acid 4, over a period of 53 days, injected every fourth day. Examined 2 days after last injection. (See protocol Rat 25–1, p. 16.) Neutral red 1:10,000. The neutral red brings out emphatic concretions.

FIG. 33. Fibroblast from thigh of same case. Neutral red 1:10,000.

FIG. 34. Drawing showing size of vacuoles in macrophage from puncture-point area of same case.

FIG. 35. Macrophage. Subcutaneous tissue from thigh of rat which has received 10 daily 1 c. c. doses of 0.5 per cent vital new red. Examined 3 days after last injection. (See protocol Rat 10, p. 18.) Physiological saline.

FIG. 36. Another macrophage from same case. Neutral red 1:10,000.

FIG. 37. Fibroblast from same case. Janus green 1:10,000. The pale-green mitochondria are easily separated from the vital-new-red deposits, even though their morphology is strikingly similar.

FIG. 38. Macrophage. Subcutaneous tissue from thigh of rat which has received six 1 c. c. intraperitoneal doses of 0.1 per cent trypan blue over a period of 16 days, injected every fourth day. Examined 1 day after last injection. (See protocol Rat 325, p. 19.) Neutral red 1:5,000. The trypan-blue deposits are seen to be almost exclusively crystalline, although there is some increase of the vacuolar segregation-apparatus as seen by neutral red.

FIG. 39. Macrophage. Subcutaneous tissue from abdomen of rat fed Niagara blue BB, the benzidine homologue of trypan blue, total 5 to 6 grams, in milk-and-egg mixture over a period of 24 days. (See protocol Rat 4, p. 19). Physiological saline. The scanty vital-dye deposits are usually deep-blue crystalline structures, many of which can be seen to have a vacuolar origin.

FIG. 40. Macrophage. Subcutaneous tissue from abdomen of rat fed for 13 days on 0.25 per cent dye T 1824 (an isomer of trypan blue) solution in milk. Examined at the close of dosage. (See protocol Rat 247, p. 20). Physiological saline. The only vital dye deposits of an appreciable intensity are crystalline "sickles."

FIG. 41. Another macrophage from same case. Physiological saline.

FIG. 42. Macrophage near blood-vessel. Subcutaneous tissue from thigh of rat which has received 11 1 c. c. intraperitoneal doses of 1 per cent solution of dye 245 (an isomer of vital new red) over a period of 27 days. Examined 1 day after last injection. (See protocol Rat 2286, p. 21.) Physiological saline. The "saturation crystals," long saffron-colored needles, are condensing without reference to the mass of vacuoles produced by the dye.

FIG. 43. Fibroblasts from thigh of same case. Physiological saline. In the majority of cases the vacuolar origin of the saturation crystal can be ascertained.

FIG. 44. Two contiguous cells, a macrophage and a fibroblast, from the subcutaneous tissue from abdomen of rat which has received four 4 c. c. intraperitoneal doses of 0.5 per cent dye T 148 over a period of 16 days, injected every fifth day. Examined 40 days after last injection. Neutral red 1:10,000.

FIG. 45. Macrophage from subcutaneous tissue from thigh of same case after treatment with a second dye. The animal received twenty-three 1 c. c. intraperitoneal doses of 0.5 per cent trypan blue over a period of 58 days. This trypan-blue dosage was started on the day following the above examination (Fig. 44). Examined 1 month after last trypan-blue injection. Neutral red 1:10,000. The trypan blue by reason of its crystallization has distorted all the previous dye T 148 vacuoles into seed-shaped structures.

FIG. 46. Fibroblast from same case. Drawn at same date as figure 45. Neutral red 1:10,000. The trypan-blue crystals are with few exceptions forming in the small T 148 vacuoles, though only a portion of the latter are chosen for trypan deposits.

FIG. 47. Macrophage. Subcutaneous tissue from thigh of rat which has received 43 daily 1 c. c. intraperitoneal doses of 0.1 per cent dye T 148, followed immediately by 5 daily 1 c. c. doses of 0.5 per cent trypan blue. Examined 15 hours after last injection. (See protocol Rat 53, p. 23.) Neutral red 1:10,000. Crystals of trypan blue are seen condensing in the peripheral portion of the vacuoles, i. e., at the vacuole-protoplasm interface.

FIG. 48. Another macrophage from same case. Neutral red 1:10,000.

FIG. 49. Fibroblast. Subcutaneous tissue from thigh of rat which has received twenty 1 c. c. intraperitoneal doses of 0.5 per cent trypan blue over a period of 57 days. Examined 2 days after last injection. (See protocol Rat 16, p. 25.) Neutral red 1:10,000. Many deep-blue crystalline deposits of the vital dye are seen and, in addition, a considerable filar segregation-apparatus stained electively with neutral red.

FIG. 50. Macrophage from same case. Neutral red 1:10,000. The cell is the seat of many crystalline vital-dye deposits. Many of the crystals are no longer associated with vacuoles.

FIG. 51. Macrophage from same case after the animal has received 16 daily 1 c. c. intraperitoneal doses of 0.1 per cent dye T 148. Examined on the day following the last injection. Neutral red 1:10,000. Almost all of the crystalline trypan-blue deposits are now inclosed by vacuoles produced by the subsequent treatment with the red dye.

FIG. 52. Fibroblast from subcutaneous tissue of thigh from same case, also after dye T 148 dosage. Neutral red 1:10,000. Only a single instance of the inclosure of a trypan-blue crystal within a T 148 vacuole can be seen; the majority of these dense-blue fibroblastic deposits are unaffected by the extensive vacuolar segregation-apparatus.

FIG. 53. Fibroblast. Subcutaneous tissue from thigh of rat which has received fifteen 1 c. c. intraperitoneal doses of 0.1 per cent trypan blue over a period of 42 days (injected every fourth day). Examined 3 days after last injection. (See protocol Rat 43, p. 27.)

FIG. 54. Macrophage from subcutaneous tissue from the abdomen of same animal after it had received 11 daily 1 c. c. intraperitoneal doses of 0.5 per cent dye T 148 immediately subsequent to the trypan-blue dosage. Examined 2 days after last injection. Physiological saline. The pink vacuoles (the larger ones are uncolored) are produced by the red dye and have almost completely dissolved the blue deposits.

FIG. 55. Fibroblast from same case, examined after the above dye T 148 dosage. Neutral red 1:10,000. The majority of the blue crystalline trypan deposits are not affected by the treatment with the red dye (dye T 148).

FIG. 56. Another fibroblast from the same case, examined after the above dye T 148 dosage. Neutral red 1:10,000. Here most of the trypan-blue crystals are seen to be inclosed in the elaborate vacular segregation-apparatus produced by the subsequent red dye.

FIG. 57. Another macrophage from the same case, examined after the dye T 148 dosage. Neutral red 1:10,000. Here the seed-like and pure crystalline trypan-blue deposits are as yet but little affected by the secondary dosage with the red dye (dye T 148).

FIG. 58. Macrophage. Subcutaneous tissue from thigh of rat which has received twelve 1 c. c. intraperitoneal doses of 0.5 per cent trypan blue over a period of 44 days. Examined 3 days after last injection. (See protocol Rat 15, p. 28.) Neutral red 1:10,000. This is a typical picture of the angular distortion of the vacuolar segregation-apparatus caused by extensive crystal production. It is somewhat more engorged with trypan blue than is usual in this case.

FIG. 59. Fibroblast from thigh of same case. Neutral red 1:10,000. A very typical picture of the crystalline deposition of trypan blue in fibroblasts; part of the segregation-apparatus was so faint in color that neutral red was needed to show its complete extent.

FIG. 60. Macrophage from subcutaneous tissue of same rat after it had received one 4 c. c. intraperitoneal dose of 1 per cent dye T 148 and one 2 c. c. dose of the same on the fifth and seventh days respectively after the trypan blue was discontinued. Examined 2 days after last injection of dye T 148. Neutral red 1:10,000. Most of the trypan-blue crystalline deposits are inclosed in the secondary segregation-apparatus created by the administration of the red dye (dye T 148).

FIG. 61. Fibroblast. Subcutaneous tissue from thigh of same case after the dye T 148 dosage. Physiological saline. It is surprising how few of the crystalline trypan-blue fibroblast deposits have been affected by the subsequent dye treatment with dye T 148.

FIG. 62. Macrophage. Subcutaneous tissue from thigh of rat which has received 8 daily 1 c. c. intraperitoneal doses of 0.5 per cent trypan blue. Examined 13 days after last injection. (See protocol Rat 222, p. 28.) Neutral red 1:10,000. The tendency has been to obliterate the usual appearance of the vacuolar segregation-apparatus altogether and to substitute substantial spear-shaped crystalline accumulations.

FIG. 63. Macrophage from the same case examined 2 days after an intraperitoneal injection of 4 c. c. of 1 per cent dye T 148 (immediately following the above trypan-blue dosage). Physiological saline. In profound contrast to the condition preceding treatment with the red dye T 148 (fig. 62); the large vacuolar segregation-apparatus created by T 148 has almost invariably inclosed the deposits of crystalline blue.

FIG. 64. Fibroblast from subcutaneous tissues of abdomen from same case, after the dye T 148 dosage. Physiological saline.

FIG. 65. Mitochondria of a fibroblast. Subcutaneous tissue from thigh of a normal rat. Janus green 1:10,000.

FIG. 66. Mitochondria of a fibroblast. Subcutaneous tissue from thigh of a normal mouse. Janus green 1:10,000.

FIG. 67. Fibroblast. Subcutaneous tissue from thigh of rat which has received 13 daily 1 c. c. intraperitoneal doses of 0.5 per cent dye 227, a brownish-red diffusible dye made by combining 1 molecule of benzidine-disulphonic acid with 2 molecules 2 amido 8 naphthal 6 monosulphonic acid, followed by seven 1 c. c. doses given every other day. Examined 1 day after last injection. Janus green 1:10,000. The blue-green mitochondria are in sharp contrast to the red vital-dye deposits.

FIG. 68. Macrophage from same case. Neutral red 1:10,000.

FIG. 69. Fibroblast. Subcutaneous tissue from thigh of rat which has received 6 daily 1 c. c. intraperitoneal doses of 0.5 per cent dye T 257 (a dye made by combining 1 molecule of o-tolidine with 2 molecules beta naphthylamine 5.7 disulphonic acid. Examined 1 day after last injection. Janus green 1:10,000. The blue-green mitochondria are in conspicuous contrast to the pale pink segregation-apparatus created by the vital azo dye. Some filar distortions of the vacuoles are seen.

FIG. 70. Fibroblast. Subcutaneous tissue of mouse which has received nine 0.5 c. c. intraperitoneal doses of 0.5 per cent trypan blue over a period of 63 days, followed after a rest of 24 days by 0.2 c. c. of 2 per cent T 148. Examined 12 days after the last injection. Janus green 1:10,000. The mitochondria occupy a rather distinct protoplasmic zone.

FIG. 71. Fibroblast. Subcutaneous tissue from thigh of rat which has received four 4 c. c. intraperitoneal doses of 0.5 per cent T 148 over a period of 16 days (injected every sixth day). Examined 1 day after last injection. Janus green 1:5,000 (half-minute exposure). The deep blue-green mitochondria are in striking contrast to the pale-pink vacuolar segregation-apparatus produced by the vital dye; filar distortions of the vacuoles are evident at several places.

FIG. 72. Fibroblast. Subcutaneous tissue from back of rat which has received nine 1. c. c. subcutaneous injections of 1.5 per cent isamine blue over a period of 28 days (injected twice a week). Examined 11½ months after last injection. (See protocol Rat 200, p. 33.) Neutral red 1:10,000. The cell is from the actual puncture area and shows strikingly how in such situations even a negative dye may have opportunity to completely fill the fibroblastic cytoplasm with an elaborate vacuolar segregation-apparatus.

FIG. 73. Small area of the areolar tissue of the thigh of a rat which has received 18 1 c. c. intraperitoneal doses of 1 per cent trypan blue over a period of 51 days (injected every fourth day). Examined 2 days after last injection. (See protocol Rat 46, p. 34.) Practically all of the deposits in both types of cell are crystalline. Physiological saline. × 160.

FIG. 74. Fibroblast from same case. Neutral red 1:10,000. This beautiful and typical picture shows the long, delicate diamond and spear shaped crystalline vital-dye deposits, which are here almost the exclusive form of the deposit in the fibroblasts.

FIG. 75. Macrophages from the same case. Physiological saline.

FIG. 76. Macrophage and fibroblast. Subcutaneous tissue from abdomen of rat which has received nine 1 c. c. intraperitoneal doses of 1 per cent trypan blue over a period of 24 days (injected every fourth day). Examined 1 day after the last injection. Physiological saline. The deposits are less thread-like here than in the case in fibroblasts from remoter areas.

FIG. 77. Fibroblast. Subcutaneous tissue from thigh of rat which has received 10 daily 1 c. c. intraperitoneal doses of 0.5 per cent dye 226, a bright-red dye made by combining 1 molecule benzidine metadisulphonic acid with 2 molecules of the 1.8 amido naphthal 4.6 disulphonic and the so-called K acid. Neutral red 1:10,000. The neutral red, a supravital stain which was necessary to bring out the complete extent of the segregation apparatus and to show occasional filar modifications of the same, has made the amethyst crystals of the vital acid dye a deep red.

FIG. 78. Fibroblast. Subcutaneous tissue from opposite thigh of same animal after it had received 9 more 1 c. c. injections of the same solution (over a period of 15 days). Examined 10 days after last injection. Neutral red 1:10,000.

FIG. 79. Macrophage from the same case (drawn at the same time as fibroblast, figure 78). Neutral red 1:10,000.

FIG. 80. Fibroblast. Subcutaneous tissue from abdomen of same rat after it had received a total of fifty-one 1 c. c. intraperitoneal doses of 0.5 per cent dye 226 over a period of 122 days. Examined 1 day after last injection. Physiological saline. The various forms and the amethyst color of the crystalline deposits of the vital dye are well shown.

FIG. 81. Macrophage. Subcutaneous tissue from thigh of rat which has received 8 daily 1 c. c. intraperitoneal doses of 2 per cent trisulfonazoblau (a deep-purple dye made by combining 1 molecule of benzidine with 2 molecules of alpha naphthol 3.6.8 trisulphonic acid). Examined 4 days after the last injection. Neutral red 1:10,000. The olive-green rhomboidal crystalline deposits of the vital dye are dispersed among the abundant vacuoles, in which the dye is pale pink in color and in part of which a minute deep-purple concrement formation has ensued.

FIG. 82. Fibroblast. Subcutaneous tissue from rat which has received 26 daily 1 c. c. intraperitoneal doses of 0.5 per cent dye T 148. Examined 1 day after last injection. (See protocol Rat 18-1, p. 36.) Physiological saline. Extensive vacuolar segregation-apparatus; some crystalline condensation.

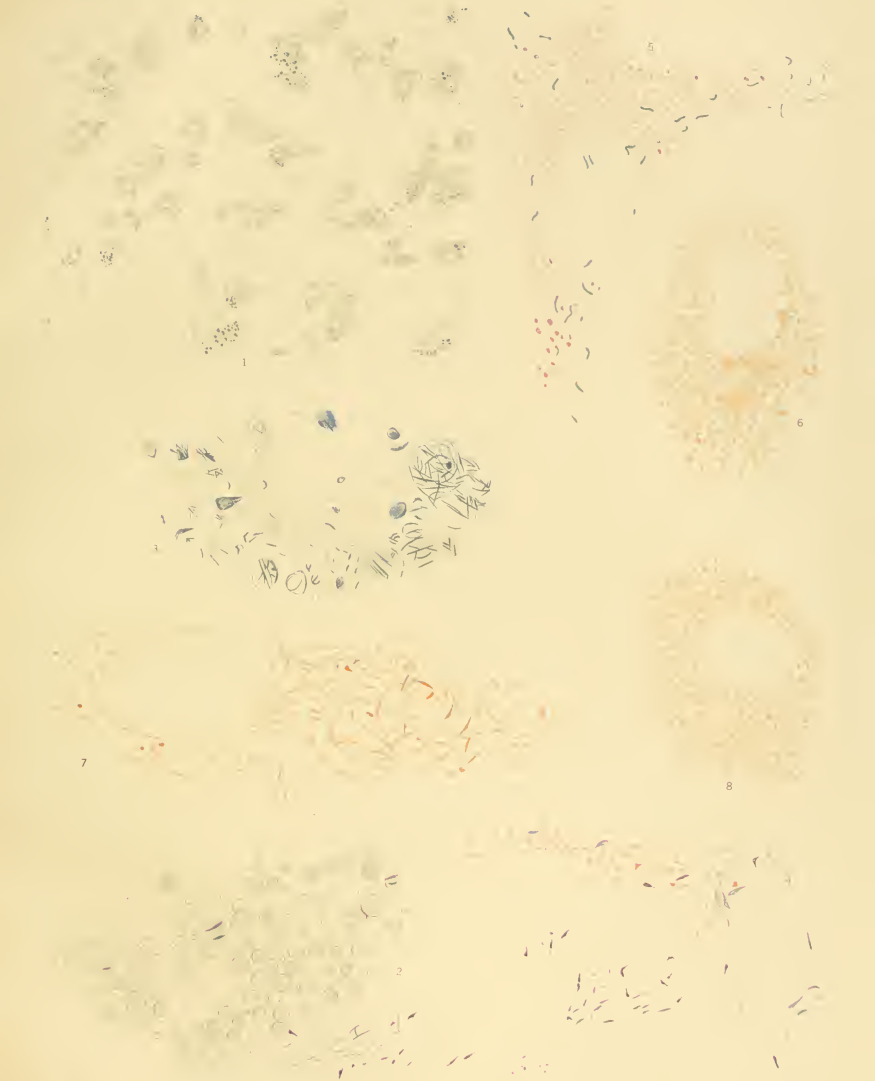
FIG. 83. Macrophage from same case. Neutral red 1:10,000. The vacuoles which house the vital dye are not greater than those characterizing the fibroblasts.

FIG. 84. Fibroblast. Subcutaneous tissue from thigh of rat which has received ten 0.5 c c.. and two 4 c. c. intraperitoneal doses of 1 per cent vital new red over a period of 56 days. Examined 8 months after the last dose. (See protocol Rat 312, p. 39.) Neutral red 1:10,000. The neutral red demonstrates that a segregation-apparatus other than that housing the deep-red permanent concretions of vital new red does not exist.

FIG. 85. Macrophage from the same case. Neutral red 1:10,000. The slight content in vital-new-red concretions when compared with fibroblasts (fig. 84) is remarkable. The neutral red shows an extensive segregation-apparatus which has lost its vital azo dye.

FIG. 86. Fibroblast. Subcutaneous tissue from thigh of rat which has received 10 daily 1 c. c. intraperitoneal doses of 0.5 per cent dye 230. Examined 3 days after last injection. Janus green 1:10,000. The mitochondria were partially degenerated at time of drawing.

FIG. 87. Fibroblast. Subcutaneous tissue from thigh of rat which has received 4 daily 4 c. c. intraperitoneal doses of 1 per cent and 2 of 0.5 per cent trypan blue. Examined 1 day after last injection. Neutral red 1:10,000. The case shows well the tendency of this dye to establish filar modifications of the segregation-apparatus.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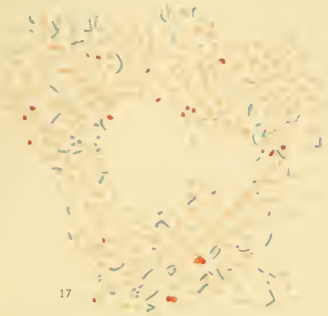
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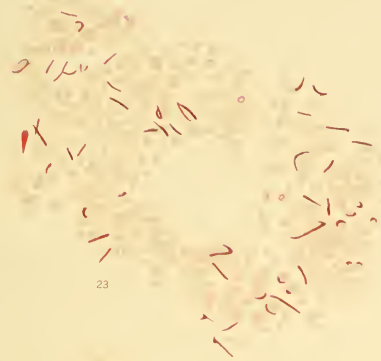
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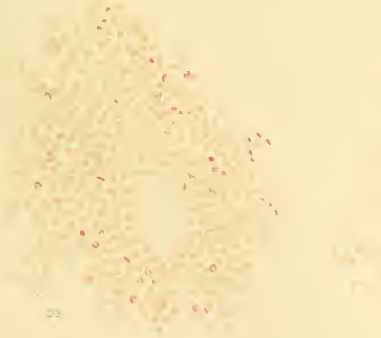
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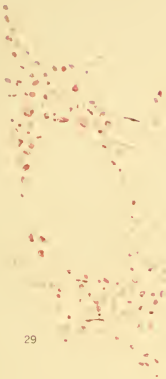
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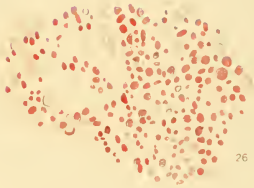
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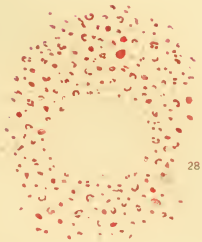
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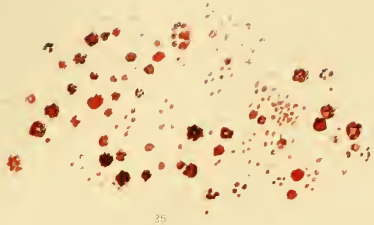
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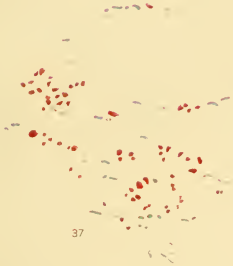
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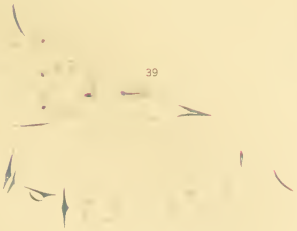
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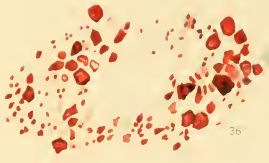
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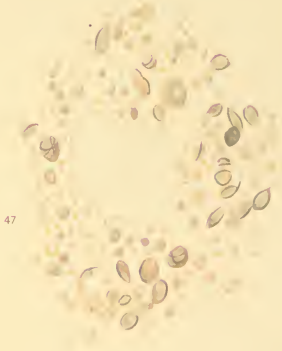

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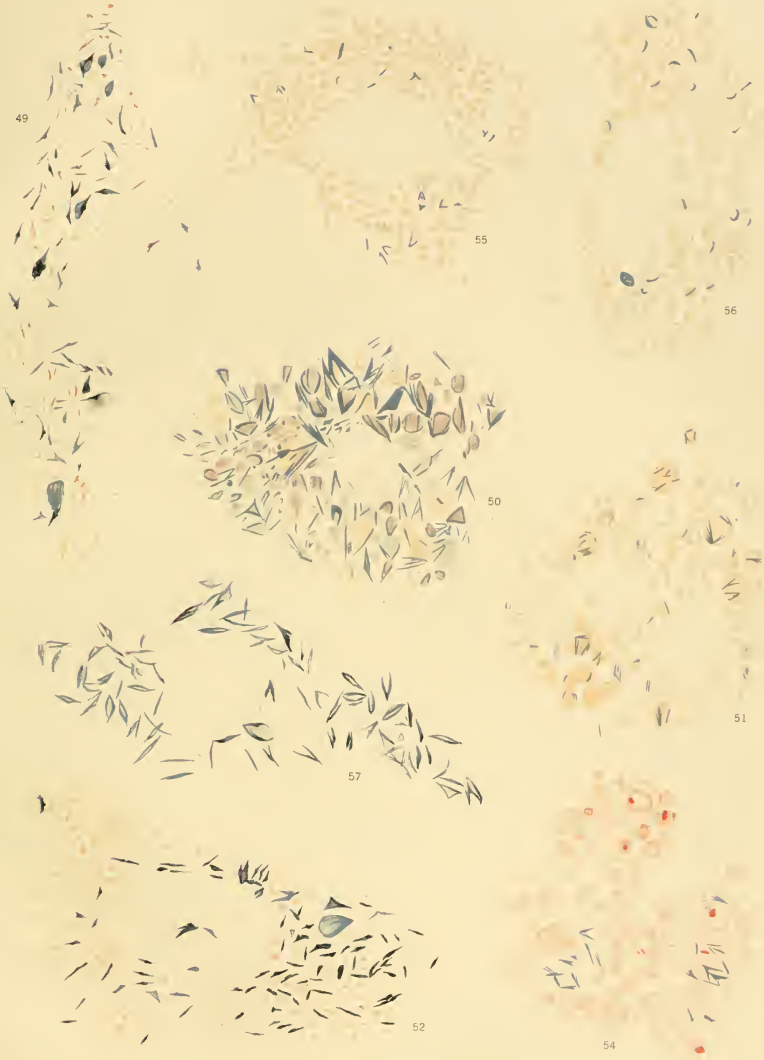
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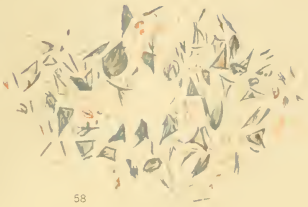

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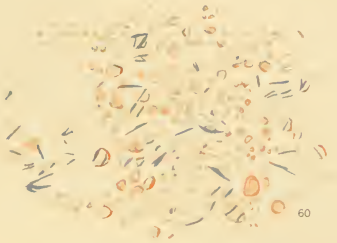

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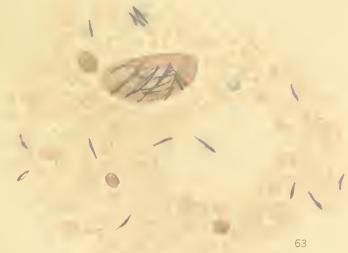

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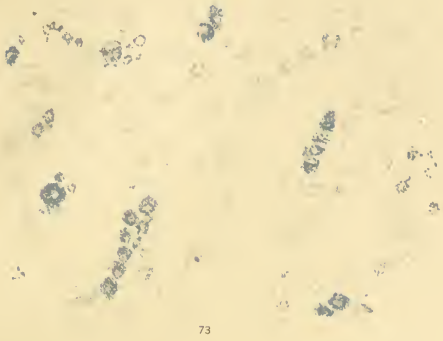

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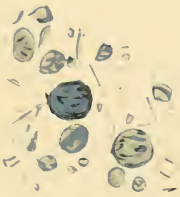

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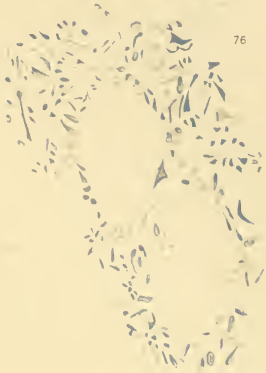

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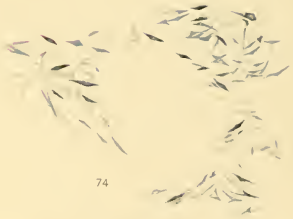

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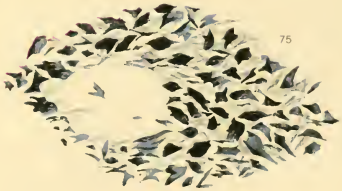

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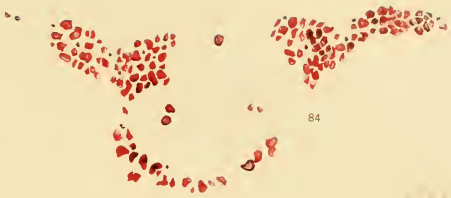
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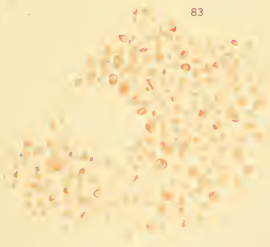
83


85

CONTRIBUTIONS TO EMBRYOLOGY, No. 48.

THE SKULL OF A HUMAN FETUS OF 43 MILLIMETERS GREATEST LENGTH.

By Charles C. Macklin,

Associate Professor of Anatomy, Johns Hopkins University.

With 5 plates containing 47 figures.

CONTENTS.

THE SKULL OF A HUMAN FETUS OF 43 MILLIMETERS GREATEST LENGTH.

INTRODUCTION.

Since the publication of my work on the skull of a human fetus of 40 mm. (Macklin, 1914) I have had the opportunity of studying and modeling a very satisfactory specimen of a somewhat younger stage. This is human fetus No. 886 of the collection of the Carnegie Institution of Washington. The sections were cut in paraffin in the frontal plane at a thickness of 100 micra and stained with alum cochineal. The technical work was all excellently done, and the series is practically perfect.

The models, of which there are 28, were made by the plaster-of-paris method of Lewis (1915), which reproduces the structures with almost absolute accuracy. The skull as a whole was first reconstructed at a magnification of 10 diameters and the details were then worked out in separate models, most of which were made at a magnification of 20 diameters. In the few instances where small details were lost, these were made good by making new models. Each model was very carefully checked by comparing each separate plate line, as the model was being painted, with the bromide photographs of the sections. The models are, I believe, as nearly exact reproductions of the original structures as it is possible to obtain. The bones were modeled on the right side only.

The parietal and frontal bones were too delicate to be modeled except in outline, and in drawing their texture the method of profile reconstruction was employed. The same method was used to check up the general outline of the skull and to obtain the relation of the external form and of the brain to the skull.

The drawings were accurately made by Mr. J. F. Didusch. The method of geometric projection was used, which insures an accurate representation of the original.

The study of No. 886 gave an opportunity for comparison of this specimen with the skull of a 40 mm. fetus from Professor McMurrich's collection, known as "I^a Toronto," and hereinafter referred to as "*Ia*," which I previously modeled. In *Ia* the measurement was crown-rump, and in No. 886 it was greatest length, and this accounts for the fact that No. 886 is considerably younger though of greater linear dimension. An opportunity was also afforded to compare the skull of No. 886 with that of No. 460 of the Carnegie collection, which has recently been modeled by Lewis (1920) and which shows the condition of the skull in a human fetus of 21 mm.

Young cartilage and precartilage, although not abundant at this stage, were included, and are mentioned where they occur.

Terms of direction in the following description are all related to the basal plate in a horizontal position. Accordingly they are, at times, at variance with the terms of orientation applying to the adult skull, particularly in the ethmoidal region, since this is usually described with the basal plate almost vertical. For the same reason the terms are often different from those used in the description of *Ia*.

DESCRIPTION.

THE SKULL AS A WHOLE.

The skull of No. 886, although considerably younger than that of *Ia*, resembles it quite closely, so that I have not been put to the task of writing such a detailed account as would have been necessary had my former article not been available. I have endeavored to avoid a repetition of my former description, and to make this article largely a comparison of No. 886 with *Ia*.

Figure 1 presents the most favorable view from above. The basal plate is not exactly horizontal, the cranial end being a little closer to the eye of the observer than the caudal. The nasal capsules are seen from an oblique direction, and hence a true concept of their length is not obtained, as will be realized when the parts are regarded from the side.

By imagining the frontal and parietal bones as having been inserted on the left side, as well as on the right, and by filling the gaps between the elements of the outer border, it is seen that the contour of the cranial cavity, from this point of view, is oval, and a little wider anteriorly, in the region of the frontal bones, than posteriorly, at the widest part of the parietal plates and otic capsules. Posteriorly this contour is made up of the cartilaginous walls of the future posterior cranial fossa; anteriorly, however, it is composed of membrane and membrane bone. The chondrocranium is divided into two unequal portions in the region of the body of the sphenoid by the superior orbital fissure—an extension of the sphenoparietal fissure. Projecting into this fissure from the body of the sphenoid is the short temporal wing.

If this figure be compared with figure 1 of *Ia* we note obvious signs of advancement in the latter, perhaps the most marked being the more developed state of the anterior end of the skull. Lewis (1920) has commented upon the relatively more rapid growth which must take place in the prechordal, as contrasted with the chordal, portion of the skull, following the stage of 21 mm. which he studied, and others have expressed themselves similarly. From a comparison with No. 460, on the one hand, and with *Ia* on the other, it is very apparent that in No. 886 development of the anterior end of the skull is going on more rapidly than that of the posterior end.

Certain features of *Ia*, such as the sharp bending of the otic capsule, suggest that the specimen from which the model was made was laterally compressed and somewhat shrunken.

In figure 2 we have the most favorable aspect of the skull base. In it the basal plate is almost horizontal, the caudal end being a little closer to the eye of the observer. The view is thus not directly antipodal to figure 1, and hence the outer contours of the entire skull, and of the chondrocranium, are slightly different. The ethmoidal region is viewed from the anterior end, making impossible an adequate appreciation of its length and that of the fissura basalis, which separates the ectethmoid from the mesethmoid. The lower jaw has been entirely cut away.

A frank view of the face is seen in figure 3, which also includes partial views of the larynx and cervical vertebræ. The gaping mouth and orbits are conspicuous features.

The skull is so placed in figure 4 that the eye looks squarely into the foramen occipitale magnum. The vertebral column is tilted a little so that its lower end is slightly nearer to the eye than the upper. The skull is not absolutely symmetrical and there is a slight deflection of the axis of the basal plate to the left, as the figure shows.

Side views of the skull are afforded by figures 5 and 6, the former including the membrane bones. The depth of the posterior cranial fossa is appreciated by looking at the dorsal portion of the chondrocranium. The hyoid, thyroid, cricoid, and four upper tracheal rings are seen. The contours of the external form, brain, frontal and parietal bones, and chondrocranium are seen from the right side in figure 7 in their normal relationship. This figure was made by profile reconstruction. The other figures show various details of the chondrocranium, and will be referred to in the description.

In general form the skull of No. 886 resembles closely the 28 mm. stage of Levi (1900), which seems to be a little farther advanced than the 30 mm. stage of Jacoby (1895).

CENTRAL STEM.

The central stem of the chondrocranium is seen from the side in figure 10, with the cut surfaces of its adnexa indicated. Its chordal and prechordal limbs meet in the region of the hypophyseal fossa, where the stem appears to have been twisted through an angle of 90°, as well as strongly bent. The chordal limb represents the basal plate of the skull, while the prechordal limb contains the body of the sphenoid, with the interorbital and nasal

septa. The lower contour lines of the two limbs, as seen in figure 10, meet at an angle of 115°, as in *Ia*. The prechordal limb in No. 460 is relatively shorter, and the corresponding angle, as measured by me on Lewis's figure 5, is 125°.

The basioccipital and basisphenoidal elements of the basal plate are united by continuity of cartilage at the undefined spheno-occipital commissure. Here the plate is thinnest, as figure 11, of the midsagittal section, shows. In this figure, too, it is apparent that the anterior end is much thicker than the posterior and that the upper surface presents a deep antero-posterior concavity, located in advance of the center.

At the junction of its anterior and middle thirds the plate is narrowed, due to the encroachment of the cochleæ. The caudal end of the curved basicochlear commissure projects outward beyond the cranial. The cut edge of this union is seen on the basal plate in figure 10, and on the cochlea in figure 15. The dorsal and ventral basicochlear grooves are not so deep as those of *Ia*.

The dorsal surface of the basal plate (figure 1) is shallowly concave from side to side throughout almost its entire extent. Anteriorly, however, this concavity becomes very narrow and indistinct as the surface rises upon the basisphenoid, and disappears at the root of the dorsum sellæ. The ventral surface presents a corresponding low transverse convexity, which falls away laterally into the ventral basicochlear grooves, flanked by the protruding cochlear portions of the otic capsules. The ventral surfaces of the cochleæ and of the basal plate lie practically in the same level and thus combine to give to the ectal surface of the base of the skull a flattened appearance in contrast to the roomy concavity of the corresponding ental surface. Posteriorly, the diverging limbs pass uninterruptedly into the primordia of the exoccipitals.

NOTOCHORD.

The notochord was modeled in relief upon the midsagittal section of the basal plate and epistropheus and is illustrated in figure 11. Commencing below, it proceeds through the body of the epistropheus and dens, emerging from the apex of the latter. The cartilage of the dens projects a little farther forward than the point of exit of the chorda and upon this cartilaginous tip the chorda rests; it then springs across the very narrow gap between the dens and the adjoining caudal edge of the basioccipital and here shows some thickening. It now proceeds along the dorsal surface of the basioccipital, in the midline, for 900 micra, being buried in perichondrium. The anterior end of this part is compressed dorsoventrally. It then traverses the plate as shown in the figure, here perforating the anterior end of the beginning ossification center for the basioccipital. Within the cartilage it is a very slender thread and at its point of exit it loses its continuity, there being here a break of something less than 100 micra. Shortly after emerging below the basal plate it comes into contact with the long, attenuated pharyngeal bursa and follows a course upon the dorsal edge of this. Especially at the extremities of the bursa it is contorted and varicose. Leaving the pharyngeal bursa, the chorda lies immediately above the epithelial roof of the pharynx for a considerable distance. It then turns sharply, as an attenuated and somewhat contorted cord, to approach the body of the sphenoid, which it enters almost at right angles to the surface. Finally, it curves forward to terminate in the body of the sphenoid near its dorsal surface and some distance short of the crista transversa. This basisphenoidal portion of the chorda is a little wider than that just outside the cartilage and its terminal end is somewhat nodular and irregular in direction.

In its course the notochord of No. 886 thus resembles closely that in Huber's (1912) human embryo J, No. 47, 32 mm. long, shown in his figure 10.

OCCIPITAL REGION.

The occipital region is a homogeneous mass of cartilage whose caudal boundary is formed by the margin of the primitive foramen magnum. The cranial boundary is marked by cartilaginous unions with the otic region and the foramina separating these. Proceeding from the front late ally and backward, we note the following commissures: spheno-occipital, basicochlear, capsulo-occipital, and occipitoparietal. Behind the basicochlear commissure a section of the boundary is formed by the posterior margin of the jugular foramen. The lateral half of this, which is thin and forms the concave lip of the sigmoid sulcus, runs almost directly outward and so makes an angle with the medial thicker and more rounded half. It is at this angle that we find the anterior end of the jugular tubercle. The boundary is then continued as the capsulo-occipital com-

missure, which curves upward and forward around the canalicular part of the otic capsule, to become directly continuous with the capsulo-parietal commissure. It is interrupted by the conspicuous capsulo-occipital foramen (figs. 5 and 6). Figure 14 shows the surface where the commissure has been severed.

OCCIPITO-PARIETAL GROOVE.

The next section of the boundary is formed by the occipito-parietal commissure, which meets the preceding at an acute angle. It joins together the squama occipitalis and parietal plate. The latter bends inward a little upon the former to make the shallow occipito-parietal groove (fig. 14), which marks the position of the commissure upon the ental surface. The sections show that the cartilage is very much thinner here, especially at the dorsal end, though not materially different in quality as compared with that above and below. As in *Ia*, there is no trace of a corresponding groove upon the ectal surface.

The ventral end of this groove is not very well defined, but may be placed just above the capsulo-occipital foramen. On the right side there is here a very small foramen. The dorsal limit of the groove is marked by the conspicuous occipito-parietal notch which separates the pointed dorso-medial termination of the parietal plate from the underlying supraoccipital element (fig. 14). Between these extremities the groove pursues a course almost directly backward, upward, and inward.

The occipito-parietal groove has been noted by Kernan (1916) as early as the 20 mm. stage. In *Ia* it was well marked and presented two foramina upon the right side and one upon the left. These foramina are not represented in No. 886, nor are they noted in No. 460 or in Kernan's specimen. Furthermore, in *Ia* the occipito-parietal notch was considerably deeper and, indeed, terminated as a narrow slit. What appeared to be degenerating cartilage cells were found in *Ia* on the right side (the sections did not include the corresponding region on the left side), uniting the parietal plate with the squama in the region of this cleft. From this it would seem that there is a gradual disunion of these two plates in progress at the posterior end of the occipito-parietal commissure during this developmental period.

The groove for the endolymphatic sac, which Lewis mentions having found upon the "masto-squamal plate," corresponds to the anterior end of the occipito-parietal groove. In No. 886 the attenuated prolongation from the endolymphatic sac lies immediately medial to the anterior end of the groove, but separated from it by 0.5 mm. In *Ia* it was similarly situated. It is not impossible that this groove may be related historically to the endolymphatic sac, for it leads backward and inward to a point quite close to the processus ascendens; and in the lizard, as has been pointed out by Gaupp (1900) and others, the endolymphatic sac lies just lateral to the processus ascendens. The region between the otic capsule and the tectum posterius has undergone an enormous amount of extension in the mammals, and particularly in man, and the endolymphatic sac has accordingly become removed from its original position above the tectum posterius, because of its connection with the otic capsule.

PROCESSUS ASCENDENS.

Of the cranial boundary there remains to be completed only that part lying between the aforementioned occipito-parietal notches. This line, which marks the thin upper border of the supra-occipital cartilage, is transverse, with a backward bowing (fig. 1). Projecting upward from this, in the midline, is a single small nodule of rather young cartilage. Upon either side of it are the fundaments of the interparietal bone, which are seen as thin strips lying close to the upper edge of the supraoccipital. This nodule is spheroidal and its anterior face projects forward a little. It is directly continuous below with the supraoccipital cartilage. This process corresponds to the "short process" of Fawcett (1910*b*), which he found in a 30 mm. human embryo. He states that it "seems to correspond exactly with the ascending process of the tectum synoticum of reptiles and amphibia as figured in Hertwig's Handbook of Embryology." In this homologization I agree. Bolk (1904) shows such a process in his figure 1, plate 6, projecting upward from the "Knorpelspange," but does not describe or name it. This figure was drawn from a human skull, apparently of about the same stage of development as No. 886. The "Knorpelspange" of Bolk, be it remarked, is merely the upper edge of the tectum posterius, which remains uncalcified, and so may be stained by the methylene blue of the van Wihje method which he uses, while the calcified cartilage below it, which Bolk erroneously interprets as membrane, is not stained in his preparations.

In the pig's chondrocranium Mead (1909) has described a single free nodule of cartilage just above the tectum posterius in the midline, which seems to correspond to the processus ascendens of No. 886. He thought it might possibly be the homologue of the processus ascendens of the tectum

posterius of the reptiles. In the lizard the processus ascendens has been described by Gaupp (1900) and by Rice (1920). Rice states that in the lizard it gives protection to the endolymphatic sacs, which lie upon either side of it. Such a function is, of course, out of the question in the skull of homo, since the endolymphatic sacs are very remote from this position.

In the skull of *Ia* I described a small free nodule, with a very minute cartilaginous fragment besi le it. These I termed the posterior cranial cartilages and suggested that they might represent the unpaired, elongated, transverse, free nodule of cartilage described by Bolk in human chondrocrania, which lay in the membrane in the midline some distance above the tectum posterius (and ascending process) in the position of the future interparietal bone and which seemed to be undergoing regression in his second stage, shown in his figure 2. On account of the fact that the sections of *Ia* were missing just behind these nodules I was unable to ascertain their exact relationship to the tectum. In No. 886 there is no cartilage corresponding to this single nodule of Bolk.

BASIOCCIPITAL CARTILAGE.

The basioccipital cartilage has been described with the basal plate. Its ossification center (figs. 1 and 2) is already indicated in a single area of cartilage undergoing the change preliminary to ossification. This area occupies practically the entire thickness of the plate, as shown in figure 11, and extends for about 700 micra antero-posteriorly. Its extremities, however, are indefinite and the cartilage for some distance in front of and behind it is of an older type than that toward the sides of the plate. Its innermost portion shows the greatest degree of change in the cartilage cells and there is a gradual transition toward the normal condition peripherally. Its anterior end (fig. 11) is traversed by the notochord, which is here much attenuated.

EXOCCIPITAL CARTILAGE.

Traced backward, the exoccipital cartilage undergoes a progressive widening together with a twisting and bending, the outer edge becoming tilted upward and the upper surface looking more and more directly inward and forward. The hypoglossal foramen shows no partition on either side. Its large and stout medial border is directly continuous behind with the thickened margin of the foramen occipitale magnum. This bar is bent downward to form the rounded occipital condyle, best seen in side views of the skull. The lateral border of the foramen is comparatively slender and is placed at a considerably higher level. It joins the anterior end of the jugular tubercle.

The *jugular tubercle* (fig. 1), which separates the hypoglossal foramen from the jugular cave lying laterally, is best marked anteriorly, where it is thin and high. As Terry (1917) remarks for the cat skull, it "presents much more the form of a ridge than of a tubercle." Passing almost directly backward, it mounts upon the rising surface of the exoccipital cartilage, where it becomes lower and broader. It terminates by curving inward and is lost upon the rounded margin of the foramen occipitale magnum at the posterior condylar notch. Later, in the occipital bone, the jugular tubercle comes to overlie the hypoglossal canal, and even reaches forward beyond it. Throughout its extent the ridge exhibits characteristic preossification changes, and we have here the beginning ossification center of the exoccipital fundament.

This center is shown in figure 1. Its posterior half is somewhat wider than the anterior and evidences the greatest amount of change in the cartilage. Here, too, in contrast to the anterior part of the center, the process involves the entire thickness of the plate, the area in the posterior condylar notch in figure 2, and better still in figure 4, showing it upon the ectal surface. Here the process has extended to the border of the foramen occipitale magnum, at the posterior condylar notch (figs. 5 and 6), thus implicating the neural arch of the occipital vertebra. In *Ia* the preossification change was farther advanced.

Medial to the jugular tubercle, the surface slopes down to the foramen magnum—more steeply behind than in front—and passes over upon the condylar surface, which here projects medially into the contour of the foramen (fig. 1). Just behind the hypoglossal foramen there is a hollow for the hypoglossal nerve.

Lamina alaris.—Lateral to the jugular tubercle is the lamina alaris, deeply grooved to form the sigmoid sulcus. Its outer edge, confluent above with the neighboring otic capsule, does not extend so far forward as the jugular tubercle. Ventrally the plate terminates as a thin concave lip, over which the sigmoid sinus empties into the jugular foramen. The edge of this lip, slightly convex anteriorly, shows a down-turned tip (fig. 10) which strikingly resembles the corresponding formation in the osseous condition. The ventral part of the lamina alaris is formed by the paracondyloid process.

The floor of the sigmoid sulcus rises steeply behind, thus bounding the jugular recess posteri-

orly. This steep area corresponds to the deep condylar fossa of the ectal surface. The recess is partially overhung anteriorly by the conspicuous posterior ampullary prominence, which gives to the region a cave-like appearance.

As in *Ia* there is on the left side, but not on the right, a small paracondyloid foramen (200 micra in antero-posterior diameter and rather less in transverse) which pierces the thin lamina alaris just lateral to the jugular tubercle. It contains only connective tissue. The corresponding area on the right side is very thin.

Paracondyloid process.—The paracondyloid process, less stout than in *Ia*, is a prominent object in figure 2, appearing as a ridge passing outward and slightly upward and forward from the condyle, and becoming more sharply marked laterally. It ends in a point which projects outward and a little downward (fig. 10), and which lies just lateral to and below the outer limit of the jugular foramen. I have already expressed myself (Macklin, 1914) as agreeing with Levi (1900) and Voit (1909) in their identification of the corresponding structure of their specimens as the forerunner of the jugular process of the occipital bone, and Lewis (1920) is in accord with this idea. Kernan (1916, p. 626) asserts that this process unde goes absorption.

It is at the transverse ridge formed by the condylar and paracondyloid processes that the occipital cartilage makes its sharp bend upward, as is seen in side views of the skull (figs. 5, 6, 10). In this way the ectal surface is divided into anterior (or basal) and posterior (or nuchal) areas. It is of interest that, at this stage, the paracondyloid process reaches laterally far beyond the joined transverse and costal processes of the underlying atlas, as is seen well in figure 4. This contrasts most strongly with the condition in the adult, where the reverse relation holds, the transverse process of the atlas overreaching the jugular process.

Kernan describes the tip of the paracondyloid process in his 20 mm. human embryo as separate from the cartilage medial to it, but attached to the basioccipital through a thin process of cartilage which passes medially and cranially in front of the hypoglossal foramen. This "thin process" (which is what I have described as the posterior border of the jugular foramen, and which is bounded behind, on the left side, by the paracondyloid foramen) he represents to be the independent costal process of the second occipital vertebra (not of the first, as I suggested), and the paracondyloid process is considered to be the caudal end of this. Thus, according to Kernan, the paracondyloid process does not represent the transverse process (and possibly the costal process) of the occipital vertebra, as I suggested (Macklin 1914). However, it should be noted that the paracondyloid process of Kernan is but the lateral free tip of the structure which I described under the same name. In Kernan's 20 mm. stage the paracondyloid process is very small as compared with its condition in No. 886 and *Ia*. Even if the tip of it does belong to the second vertebra, it would still seem that the main mass of it is to be looked upon as having been derived from the transverse and possibly the costal process of the occipital vertebra. Kernan notes that there is no independent costal element of the occipital vertebra.

The paracondyloid process of Kernan's specimen is directly continuous above with the lateral portion of the lamina alaris, which appears upon the ectal surface as the lower end of the formation which I have termed the crescentic ridge. Kernan erroneously speaks of this as "the inferior nuchal line of the ex-occipital portion of the adult bone." This lateral portion of the lamina alaris shows, according to Kernan, alternating interruptions and junctions with the cartilage lying medial to it and is looked upon as a costal bar, the junctions representing the bases of transverse processes of vertebræ. He suggests that "in the lamina alaris are represented the costal and transverse processes of several vertebræ. The outer bar represents the fused costal elements and may be termed the costal bar." He claims to be able to discern parts of three vertebræ in the lamina alaris.

Lewis, who describes a stage of the human embryo (21 mm.) almost identical with that of Kernan, says nothing as to this complicated structure of the lamina alaris nor as to the independence of the tip of the paracondyloid process. He states (p. 317): "The occipital transverse process forms part of the caudal and lateral margins of the jugular foramen and continues up into the squama and alar lamina without line of demarcation." Further (p. 318) he says: "The transverse or jugular process springs from the occipital hemiarch at the junction of the roots and lamina and projects laterally back of the jugular foramen. We have already noted its serial relationship with the vertebral transverse processes. The lateral extremity of the jugular process has a knob-like enlargement, and into this are inserted the rectus capitis lateralis muscle and the occipito-mastoid muscle."

It should be remarked that the structure which Kernan in his plate 3 labels as "Processus costalis et transversus, occipital vertebra" is labeled by

Lewis as "occipital condyle" in his figure 6. I have examined the Lewis model and agree with his labeling of the occipital condyle. It would seem that Kernan has placed his direction line for the occipital condyle much too near the midline and that the occipital condyle is to be found at or near the point designated by him as the "Processus costalis et transversus, occipital vertebra." The relation of the articular surface of the atlas in Kernan's sections seems to favor this view. It is quite obvious, too, that in his plate 3 Kernan has mislabeled the hypoglossal foramen, calling it the "foramen jugulare." If his labeling is correct, then the "rib element, 2nd occipital vertebra" passes in front of the jugular foramen—a relationship which is evidently impossible.

In his description and figures of the cat's chondrocranium, Terry (1917) has apparently included the "costal bar" of Kernan with the paracondyloid process, considering the process to be even more extensive than I have conceived it to be.

SUPRAOCCIPITAL CARTILAGE.

Behind the lamina alaris the squama occipitalis, the right side of which is seen from within in figure 14, becomes progressively wider to the region lying between the upper extremity of the foramen magnum and the anterior end of the occipitoparietal commissure; after this it becomes somewhat narrower. Its surfaces are fairly smooth, but there are certain markings which should be mentioned.

Just within the capsulo-occipital commissure is the medial capsulo-occipital groove, deepened in front by the posterior end of the otic capsule, around which it curves. Posteriorly this groove is bordered, in places rather indefinitely, by a low ridge, which becomes confluent below with the jugular tubercle. Just behind the capsulo-occipital foramen this ridge is quite well marked. The groove contains the transverse sinus which, when traced from above, descends until it reaches the sigmoid sulcus, where it turns sharply and runs forward for a short distance before plunging downward again into the jugular foramen. The corresponding lateral capsulo-occipital groove is indefinite.

The *lateral occipital eminence* (figs. 4, 5, 6) is not so conspicuous as in *Ia*, nor is the cartilage of the plate here so thick. Behind it the ectal surface shows a wide, shallow but distinct groove, limited below by the crescentic ridge, bordering the U-shaped area of cartilage around the superior incisure; the latter, in turn, protrudes slightly, as figure 4 shows. The ends of this low ridge are confluent with the extremities of the crescentic ridge in the region of the dorsal foraminal prominences. Both shallow groove and low ridge are represented upon the ental surface in reverse, as shown in figure 14.

The *crescentic ridge* or crista arcuata occipitalis (figs. 2, 4, 5, 6) sweeps upward, backward, and inward from the tip of the paracondyloid process to end rather indefinitely in the region of the dorsal foraminal prominence. Its structure is very similar to that of *Ia*. The ventrolateral end is narrow, raised, and distinct, but as the ridge passes backward it broadens and flattens out.

Judging from his plate 2, Kernan has divided the structure which I termed in *Ia* the crescentic ridge into two parts, the dorsomedial of which he labels "crescentic ridge" and the ventrolateral, "costal bar, lamina alaris." I used the term "crescentic ridge" (as I have done in No. 886) to apply to the entire ridge, beginning at the tip of the paracondyloid process and ending upon the border of the foramen occipitale magnum. Kernan says that this structure is the inferior nuchal line. I think it will be obvious to anyone who examines the models of the chondrocranium showing these ridges and compares them with the mature skull, that this interpretation is not correct, for, as is well known, the inferior nuchal lines, although arising anteriorly from the same region, viz, the jugular process, yet meet dorsally upon the external occipital crest at a point some distance behind the posterior margin of the foramen occipitale magnum. In the models, on the contrary, the ridges do not meet, but end upon the margin of the foramen, at the dorsal foraminal prominences, which are situated some distance anterior to the posterior limit of the primitive foramen. Now, if the primitive foramen closes by the approximation of the dorsal foraminal prominences (which represent the tips of the neural hemiarches of the occipital vertebra, as described in my former paper), then these crescentic ridges will meet upon the posterior border of the foramen, and not some distance behind it, as do the inferior nuchal lines. If, on the other hand, the dorsal foraminal prominences do not fuse in the final closure of the foramen, then these crescentic ridges will never meet, but will end upon the foraminal margin. Thus their posterior extremities can not be made to coincide with those of the inferior nuchal lines. It does not seem possible that in the closure of the primitive foramen more of its area will be taken up than that of the wide superior occipital incisure (lying above and behind the dorsal foraminal prominences), judging from my later stage and that of

Hertwig. Hence it would seem quite likely that the crescentic ridges never come to occupy the position of the inferior nuchal lines, and hence they can not represent them.

In the mature occipital bone the crescentic ridges are represented rather imperfectly, but are nevertheless recognizable as the lateral delimitations of the condylar fossa. Their lateral extremities are here fairly distinct, reaching the jugular tubercle, but their posterior extremities are lost in the region of the posterior margin of the foramen magnum.

The paraforaminal area, or condylar fossa, is the depressed area bounded by the crescentic ridge, the paracondyloid process, and the occipital neural hemiarch. Its size here is relatively much greater than that of the corresponding region of the mature bone. As yet the superior articular process of the atlas does not lie far enough out, as figure 4 shows, to reach the fossa. Its floor is unperforated, but is very thin. The corresponding convexity upon the ental surface forms the posterior boundary of the jugular recess.

The supraoccipital fundament includes the dorsomedial parts of the two squamae, joined together above the superior occipital incisure by the tectum posterius. The tectum is practically plane and its ental surface looks almost directly forward. Its lower border presents a descending process, which projects into the superior occipital incisure (figs. 4, 14). It is of a younger type of cartilage than that of the tectum above it. In a 30-mm. human embryo a similar process has been described by Fawcett (1910*b*). The ascending process has been described. The cartilage of the tectum is very thin, as is seen in the midsagittal section shown in figure. 14.

The ossification center for the supraoccipital is single at this stage. Its right half is seen from the front in figure 14, and figure 4 gives a view of it from the rear. It is somewhat butterfly-shaped and terminates laterally in sharp down-turned points which do not pass beyond the occipitoparietal commissure. From these lateral extremes the outer border curves downward and inward to the dorso-lateral angles of the superior incisure of the foramen magnum. The ascending and descending processes are not included in the ossification, which, however, involves the entire remaining tectum posterius with the exception of a narrow edge along the superior border. No actual bone has yet been deposited, but there has evidently been considerable calcification of the cartilaginous matrix, judging by the staining reaction.

It is quite evident that this area of calcified cartilage has remained unstained in Bolk's (1904) van Wijhe preparations of the occipital region of the human skull, and he has accordingly misinterpreted this region as membrane. This failure to recognize an imperfection in his method has led Bolk into a number of errors. Thus he is in error when he states that it is highly improbable that the supraoccipital ossification is of the true endochondral type. It is as distinctly endochondral as any ossification could be. This region is unique in that the calcification of the cartilage takes place very early. From his preparations Bolk gains the impression that bony development begins in the center of the supraoccipital before cartilaginous development is accomplished here, the failure to chondrify being due to the rapid development of the brain. He does not explain why this rapid brain development should not also have a similar effect in retarding the development of the "Knorpelspange" just above, which is merely the uncalcified upper margin of the tectum posterius, as I have pointed out.

It is of interest, however, that this upper margin does remain uncalcified and unossified for such a long period for, as Bolk has shown, it is present throughout his series of four human chondrocrania. In the last, however, it is becoming thinner and more attenuated. I would suggest that this cartilaginous edge may be retained to favor growth of the supraoccipital here. A similar reason may underlie the persistence of the twin nodules of cartilage which are present at the apex of the superior occipital incisure, in Bolk's preparations, and which he states agree in position, in one case at least, with the bones of Kerckring. These nodules, in Bolk's figure 1, are in the same position as the descending process in No. 886, apparently. It seems to me quite likely that they are not isolated masses of cartilage situated in membrane, but that they are connected with the calcified cartilage of the tectum above.

It is of interest to observe that Fawcett (1910*b*) questions Bolk's findings when he says (p. 306): "I must confess the appearances in his figures scarcely explain what is seen in this cranium." Some other recent authors have not been quite so critical.

OCCIPITAL VERTEBRA.

The neural hemiarch of the occipital vertebra does not stand out so distinctly as that of *Ia*. Behind the condyle it is bent to form the posterior condylar notch. In figure 4 it is quite distinctly

outlined, terminating in the dorsal foraminal prominences. The ental surface shows, just lateral to the hemiarch, a groove which is rather indefinitely marked except just behind the jugular tubercle. The dorsal foraminal prominences are much farther apart than in *Ia*, and the foramen occipitale magnum is correspondingly larger. There is, throughout the entire extent of the hemiarch, a direct connection of its cartilage with that of the adjoining squama. In the region of the aforementioned preossification center its material is of a distinctly more advanced type than that of the neighboring squama.

It is of interest to note that Lewis has found the tip of the arch, in a 21-mm. human embryo, separated from the squama, this tip projecting dorsally into the mesenchyme. Kernan (1916) does not mention this separation in his 20-mm. human embryo. In three other human embryos examined by Lewis, of 20 mm. length, there was no separation of the occipital hemiarch from the squama, though there was a difference in the character of the cartilage of these parts, the squama being of a younger type. Even in the 19 mm. stage, Lewis found a greater amount of fusion between these structures than in No. 460, so that there seems to be some variation here. No. 460, it may be noted, was a negro skull. Because of this separation of the occipital neural hemiarch from the squama in No. 460, and of the difference in the character of the cartilage here and in the other embryos examined, together with his observation that "there is also a more gradual transition as regards the degree of differentiation from the cartilage of the transverse process into the squama than from that of the lamina," Lewis favors the view that the squama arises by upward extension from the transverse process of the occipital vertebra rather than from the occipital neural hemiarch, as I formerly suggested.

FORAMEN OCCIPITALE MAGNUM.

The foramen occiptale magnum is relatively larger than in *Ia*. Its lateral contour shows the dorsal and ventral foraminal prominences (figs. 5, 6). Seen from without, the plane of the intercondyloid incisure looks downward. The region lying between the prominences faces almost directly backward and also a little downward. Viewed from the side it is seen to present a distinct dorsal concavity, corresponding to the posterior condylar notches. At these points the foramen is widest (fig. 1). The superior occipital incisure, whose plane is directed backward and slightly upward, is filled with the spino-occipital membrane. It is much wider than in *Ia*. The superior occipital incisure may persist as a well-marked notch in adult skulls of certain dogs. Dr. A. H. Schultz has shown me three very striking examples in skulls of bull dogs and pugs. These are Nos. 71, 381, and 382 of the Schultz collection.

CERVICAL VERTEBRÆ.

The cervical vertebræ (figs. 3, 4, 5, 6) are well developed. The distance between the tips of the hemiarches of the atlas is the same as that between the dorsal foraminal prominences, but below that the hemiarch tips gradually become more closely approximated, those of the seventh vertebra being separated by a comparatively short interval. A comparison with Lewis's figures of the same region gives a graphic demonstration of the closure of this part of the spinal canal and foramen magnum.

The *atlas* presents a distinct anterior arch or hypochordal bar, which is separated from the dens epistrophei by a thin sheet of connective tissue (fig. 11). The late al masses are stout and show concavities upon the upper surface for the condyles of the occiput. The costo-transverse foramen on the left side is closed, but that on the right side lacks a very short piece of the costal process which, however, appears to be forming.

The *epistropheus* presents a stout dens, from whose tip emerges the notochord, as shown in figure 11. The bodies of the vertebræ form a line which is almost straight and which makes with the basal plate an angle of 125°. The corresponding angle in the Lewis 21-mm. embryo was 110°, as measured by me from his figures. This angle is probably variable.

TYPE OF CARTILAGE.

With the exception of the ossification centers, which have already been described, the occipital region is made up almost entirely of a mature type of cartilage. The character of this varies somewhat in the different regions. The paracondyloid process is tipped with young cartilage. The condyle, too, reveals a younger type of cartilage at the region of the future articular surface.

OTIC REGION.

PARIETAL PLATE.

The parietal plate (figs. 1, 5, 6, 14) is thin and slightly concave entally. Its junction with the squama occipitalis has been mentioned. Anteriorly it is connected with the otic capsule by the capsulo-parietal commissure which is interrupted by the large and elongated capsulo-parietal foramen. The plate, above the commissure, bends outward over a groove which is bounded below by the otic capsule.

The dorsalmost, irregularly rounded part of the plate is the highest part of the chondrocranium and is partially cut off from the main portion by distinct notches, most closely approximated on the right side. Its anterior edge does not overlap the developing parietal bone, as in *Ia*, but is separated from the posterior edge of this bone by a very narrow interval (fig. 5). There are no small cartilaginous remnants above it, as in *Ia*; indeed this portion of the plate shows a relatively greater development than in *Ia*, or (even more pronounced) than in the Hertwig model, in both of which it is evidently undergoing reduction.

The anterior extremity of the main portion of the plate is rounded and does not project far beyond the capsulo-parietal commissure. Posteriorly the plate curves inward, gradually narrows, and ends in a point, separated from the supraoccipital cartilage by the occipitoparietal notch. The upper edge of the entire plate is rather rough.

DORSAL TECTA.

The idea that there are two tecta represented in the dorsal part of the occipital region has recently been given attention by several authors. Kernan (1916, p. 620) regards the tectum synoticum, which joins the otic capsules through the parietal plates, as a primitive structure which is "formed early, and is absorbed as the tectum posterium reaches its development." The tectum posterius joins the occipital wings and thus completes the foramen occipitale magnum. This view Kernan arrives at from a comparison of the findings in his 20-mm. human embryo with those of my 40-mm. human embryo, together with a consideration of the earlier evidence brought forward by Levi. In his specimen Kernan describes the parietal plates (which are marked off from the underlying occipital wings by the occipito-parietal grooves) as being joined behind to form a true cartilaginous tectum synoticum. In this way the primitive foramen magnum is completed, for the occipital squamæ are not yet in union. In *Ia* the parietal plates, although stretching inward toward one another, do not unite, while below them the occipital wings are united in the tectum posterius. Levi, according to Kernan, "showed that the dorsal union between the two sides occurred cranially and advanced caudally and ventrally, the more cranial union between the parietal plates being absorbed as the ventral union is formed." Thus Kernan remarks: "The conditions in Macklin's 40-mm. and this 20-mm. embryo would appear to bear out this statement of Levi." This idea has been enunciated by Rice (1920, p. 137).

Fawcett (1918*a*, p. 227) writes: "In man there are two tecta (Bolk, Fawcett): one a very wide one, the more posterior, therefore called the *tectum cranii posterius*, from the middle of whose anterior border a processus ascendens arises (Fawcett; in pig, Mead); the other, the *tectum cranii anterius*, is very slender and quite isolated, not reaching the parietal plate on either side, nor being in any way connected with the tectum cranii posterius. Recently I have observed two tecta in the cat: one certainly the ordinary tectum posterius, the other small, median, and anterior to this, which may be an isolated processus ascendens or may be looked upon as a tectum anterius." In Weddell's seal Fawcett (1918*b*) reports two cartilages, one on either side of the midline, situated very far forward, and belonging to the anterior tectum. They showed slight signs of fusion with one another.

The condition in No. 886 does not add anything to the information given by *Ia*, the parietal plates ending as free points projecting into the dorsal membrane. It is possible that the most anterior cartilage of Bolk (1904) is to be looked upon as a rudiment of the link which once joined the parietal plates and, if this be so, then it is possible that the posterior cranial cartilages of *Ia* belong to this band.

OTIC CAPSULE.

In addition to the connections of the otic capsule already noted, there is a small cartilaginous union with the processus alaris, one with the incus, and one with the styloid process.

As Lewis remarks for his 21-mm. specimen, the capsule is placed in about the same position, with regard to the basal plate, as is the petrous portion of the temporal bone. Its shape, roughly that of half a pear (with the large end situated dorsolaterally and the cut surface toward the cranial

cavity), is best appreciated from a study of the figures. Its walls, except at certain regions to be described, are thin.

Cranial surface.—The entire cranial surface of the capsule is seen in frank view in figure 17. The pars cochlearis is here quite smooth and flattened, and presents the wide internal acoustic meatus. The more irregular pars canalicularis shows distinct rounded eminences for the superior and posterior canal spaces, above which is the groove for the transverse sinus. The relation of this surface to the inner cavity is ascertained by a comparison of figures 17 and 15. The capsule was drawn from the same viewpoint in the two cases, but in figure 15 most of the medial wall has been removed. Above the space for the superior canal is the cut edge of the capsulo-parietal commissure, interrupted by the long capsulo-parietal foramen. The posterior slender limb of this commissure is joined to the angular mass of the capsule. Proceeding downward, we note the connection with the squama occipitalis, broken by the large capsulo-occipital foramen. The cut edges of these unions are seen in figure 14. Their line of attachment is along a rounded border separating the medial and lateral surfaces of the pars canalicularis. The subarcuate fossa, overarched by the superior semicircular canal, is very deep. It contains only loose connective tissue and small vessels. Just behind it, in the prominence for the crus commune, is the endolymphatic foramen. It is wide and elongated and has very thin borders of young cartilage. The ductus endolymphaticus, traversing the foramen very obliquely, fills but a small fraction of it, the remainder being completed by membrane. The relationship of the transverse sinus to the endolymphatic duct and sac of this embryo may be seen in plate 5 of an article by Streeter (1918). In *Ia* the endolymphatic foramen was narrower and had thicker walls; there was a short process projecting dorsally from the upper lip, which is not present in No. 886.

Behind the endolymphatic foramen is a sharp spur of cartilage which projects inward and backward medial to the transverse sinus. It is a short distance below and behind the endolymphatic sac. Underlying it the wall is quite thick, so that the posterior canal is removed some distance from the surface here. The inferior ampullary prominence is conspicuous below. It terminates in the posterior intraperilymphatic process, which projects downward into the perilymphatic foramen.

Lateral surface.—The lateral surface can not be satisfactorily seen from one standpoint. Figure 20 presents the most comprehensive view. From below upward may be distinguished the cochlear vestibular, and canalicular areas.

The cochlear area, looking principally downward, is very convex and bulging. It shows a very shallow furrow, the septal sulcus, which has the form of a helix and is very indefinite towards the pole (fig. 20). It corresponds to the line of attachment of the spiral septum upon the interior of the capsule (fig. 15). The internal carotid artery lies in a short length of this groove, as seen in figure 2. Turning upward around the cranial pole of the cochlea, it traverses the lateral side of the carotid foramen, keeping close to the alicochlear commissure, and thus gains the cranial cavity. Just lateral to the ventral basicochlear groove is a long prominent rounded ridge, formed by the space for the first turn of the cochlear duct. This terminates posteriorly in the promontory; anteriorly it turns upward to end at the rounded ventral pole of the cochlea. Somewhat below the apex of the pole (figs. 1, 6, 20, 23) is the slender alicochlear commissure.

There is, at this stage, no evidence of the small supracochlear cartilage, which I described in *Ia* just above the cranial pole of the cochlea and below the semilunar ganglion. Its position in *Ia* is somewhat above that of the union of the alicochlear commissure with the cochlea, found in No. 886.

The vestibular area is sharply marked off medially by the rounded contour of the cochlea and is laterally blended with the inferior canalicular surface. It shows, adjoining the promontory, the large vestibular window. Above is seen the facial foramen, surmounted by the suprafacial commissure. Across this area the facial nerve courses (fig. 16), lying close to the cartilage. The great superficial petrosal nerve is seen leaving the geniculate ganglion.

The canalicular area is crossed by a ridge which, beginning above at the capsulo-parietal commissure, runs downward over the tegmen tympani and then backward and downward over the parotic crest to end at the dorsalmost extremity of the jugular foramen. Medial to this ridge the surface looks principally downward and extends inward as far as the inferior ampullary prominence. The major portion of the canalicular surface, lying behind and above the ridge just outlined, looks directly outward (figs. 5, 6). It is somewhat convex, especially from before backward. All of the canals make prominences, but these are all very low and rather indefinite. The central and superior, almost plane, portion of the surface belongs to the angular mass, a large lump

of cartilage (figs. 24, 25) which is inclosed by the semicircular canals. A small area in front of the anterior limb of the lateral semicircular canal has failed to chondrify fig. 20, *o*). It is not seen in later stages.

The *fenestra vestibuli* (fig. 20) is roughly oval in contour, with a narrow anterior extremity. Its ventral border, which is formed by the upper free edge of the promontory, is almost straight. The edges are thin, and of a young type of cartilage, which is indefinitely merged with the membrane which fills it. This membrane, which represents the annular ligament, bulges into the cavity of the capsule, as shown in figure 15. It resembles precartilage and its inner surface is cellular and heavily staining. The stapes occupies only a small fraction of its area. A slender isthmus of cartilage separates the fenestra vestibuli from the fenestra perilymphatica, and from this a low ridge runs directly backward to the lateralmost end of the jugular foramen.

The *fenestra perilymphatica,* at the caudal extremity of the cochlea, looks directly backward, and hence can not be shown in the ordinary views, but in figures 5, 6, 17, and 23 arrows are used to indicate its position. Its margin has the form of an irregular ring, sharply bent upon itself, the bent parts being situated (laterally) at the commissure separating the two fenestrae, and (medially) at the inner corner of the foramen, which will form the cochlear aqueduct. The parts of the ring which approach one another are the posterior intraperilymphatic process, situated above and posteriorly (in my former article referred to as the interperilymphatic process) and the anterior intraperilymphatic process situated below and inferiorly. The latter is a small ridge of cartilage which appears upon the inferior border of the fenestra. It seems to correspond to that described by Terry in the cat, and marked with an asterisk in his figures 2 and 12. These two points are apparently growing together, as shown by later stages. In *Ia* the posterior process was somewhat longer. Thus is accomplished the partitioning of the perilymphatic fenestra into the cochlear fenestra laterally and the cochlear aqueduct medially, The medial half of the border is seen in figure 23.

Jugular foramen.—The posterior margin of the jugular foramen (fig. 10) has already been described with the occipital region. The anterior margin, lying at a higher level, is seen in figures 15 and 17 between the basicochlear and capsulo-occipital commissures. This border is bent downward by the inferior ampullary prominence and posterior intraperilymphatic process. Between this process and the anterior intraperilymphatic process is a gap in this border which will be completed by the union of these processes.

The intracranial ganglia of the glossopharyngeal, vagus, and accessory nerves are continuous and form a conspicuous object just above the level of the jugular foramen. This chain of nerve cells lies almost directly antero-posteriorly, with a slight medial inclination of the caudal end, and crosses the jugular tubercle, which it almost touches. The jugular ganglion of the vagus is the largest element of the chain; it has the form of a ring, the central part being composed almost entirely of fibers. The outer edge of this ring, situated at a higher level than the inner, lies just medial to the inferior ampullary prominence. In front of this ganglion, and a little above the site of the cochlear aqueduct, is the jugular ganglion of the glossopharyngeal. The accessory portion of the chain is long and thin. The petrous ganglion of the glossopharyngeal is situated close behind the lower border of the perilymphatic foramen, its upper limit reaching as high as the anterior intraperilymphatic process. It is larger and thicker than the jugular ganglion of the same nerve. Between its ganglia the trunk of the glossopharyngeal nerve runs downward and slightly outward just medial to the line joining the intraperilymphatic processes. The ganglion nodosum of the vagus is a much larger structure. It is very much elongated, with attenuated upper end, which reaches to the lowermost limit of the petrous ganglion but lies slightly medial to this. Its main direction is downward, with an appreciable inclination backward.

The *suprafacial commissure* is seen in figures 17 and 20, and the cut surfaces, after the removal of the commissure, appear in figures 15 and 16. It connects the pars canalicularis just above the region of the recessus ellipticus with the superior border of the cochlea. It is flattened from above downward and is a little narrower below than above. The edges are quite thin. The relation to the facial nerve is seen in figures 15 and 16. In Kernan's specimen the commissure was incomplete, but it was complete in Lewis's No. 460.

The *tegmen tympani* (fig. 20), which overlies part of the developing ossicles, is a well-marked ridge of cartilage projecting forward from the superior ampullary prominence to form the most anterior part of the pars canalicularis.

The *crista parotica,* though less prominent than in *Ia,* is quite conspicuous and forms a ridge lying between the hole (fig. 16, *o*) in the capsule and the mastoid process. Just above its anterior end there is a small area for union wth the crus breve

of the incus. Its posterior portion is concerned with a union with the styloid process (figs. 5, 6, 16). The facial nerve courses under the shelter of the ridge (fig. 16) in the site of the future facial canal.

The *styloid process* (figs. 4, 5, 6, 42, 44) is attached to the otic capsule, thus differing from the condition in *Ia*, where it was separate. In figures 42 and 44 of the process and figure 20 of the capsule the division has been made higher up than one would think should be the case, judging from figure 16, and it would seem that a little of the crista parotica has been excised and appears as the upper expanded end of the process in figures 42 and 44. There is along this line of section, however, a layer of lighter-staining cartilage which was used as a guide in making the division. At the 20 mm. stage Kernan states that the styloid process is "received in a depression of the cartilage" at the dorsal extremity of the crista parotica.

The first part of the process passes almost directly inward, with a slight inclination downward, and approaches quite close to the capsular wall, which here rises slightly as a very low narrow ridge. Thus we have almost a complete primitive stylo-mastoid foramen for the exit of the facial nerve, the relation of which to the styloid process appears in figures 42 and 44. The styloid next sweeps downward around the posterior edge of the developing tympanic cavity, forming a curve with a strong backward convexity. It then passes into the terminal long, tapering, and almost straight part which is directed downward, inward, and forward. It shows a membranous connection with the lesser cornu of the hyoid (figs. 42 and 44).

The *facial nerve*, after passing around the styloid process, turns downward and then forward, and breaks up into the usual branches (fig. 42). From the lateral aspect of the concavity thus made, and but a short distance below the root of the styloid, the chorda tympani is given off (fig. 16), which passes almost directly forward, just above the superior extremity of the tympanic cavity and between the crus longum of the incus and the manubrium of the malleus, after which it turns downward and runs also a little forward and inward, crossing the line of the tympanic cavity and tuba auditiva, but diverging from these to reach the lingual, as shown in figures 31 and 34.

The *mastoid process* of the right side is a small nodule of young cartilage separated from the capsule by perichondrium. Its shape is that of a short rod, about 300 micra long antero-posteriorly. The nodule on the left side is similar. In *Ia* the process was connected with the otic capsule by cartilage. In No. 460 it was somewhat longer, relatively, than in No. 886, but there it consisted of blastema. Lewis found, in other embryos of about the same stage as No. 460, that a separate cartilage in the blastema of the mastoid process was present. He notes the attachment of the digastric and stapedius muscles to the process. Kernan notes the process in his specimen.

Lewis describes the "*mastoid cartilage*" to which the mastoid process is attached. In his models it is a flange of cartilage which is attached to the caudal and dorsal border of the pars canalicularis. In No. 886 the mastoid process is attached to the pars canalicularis itself, a short distance in advance of the posterior end of the posterior semicircular canal. It would seem that part, at least, of this mastoid cartilage of Lewis is later taken up into the caudal end of the pars canalicularis. In No. 886 the region corresponding to the mastoid cartilage of No. 460 is very much less prominent.

Spiral septum.—Looking into the interior of the cochlea (fig. 15) the spiral septum is seen. In my former article it was referred to as the spiral lamina. It springs above from the posterior edge of the roof of the cochlea (which also forms the floor of the facial canal, as in fig. 15), and shows here a slender connection posteriorly with the adjacent capsular wall, just medial to the impression for the recessus ellipticus. In this way is formed the transverse crest (fig. 17). Terry describes a similar formation in the cat. Just below this union is a short spur of young cartilage (fig. 15) which projects backward under the anterior extremity of the sacculus.

This medial part of the septum is much the highest; it diminishes rapidly in height as it passes downward and forward (fig. 15). It makes a conspicuous impression upon the cast of the cochlear cavity, as seen in figure 21. As yet the entire septum shows scarcely one turn, and the second (outer) portion of this (rather less than one-half) is not at all prominent; it gradually becomes obliterated in the depths of the cave which is thus cut off. The edge is of a young type of cartilage, which is evidently growing rapidly.

The first portion of the cochlear canal space lies medial to the high first portion of the spiral septum, and upon the corresponding region of the medial wall of the cochlea there is a very low ridge (fig. 23), also edged with young cartilage. This is seen in the model of the space (fig. 18) as a shallow groove which, beginning caudad above this perilymphatic foramen, is continued forward and finally forward and upward around the medial acoustic meatus to lose itself in front of this opening. This ridge is less developed than in *Ia*, where the posterior portion of it was referred to as

the "pyramidal mass." The spiral septum and this ridge seem to be approaching one another over the cochlear duct and spiral ganglion and it is probable that in this way the first portion of the cochlear canal space is cut off—indeed it would seem that the canal coils are gradually separated in this way. As yet, however, there is a wide interval separating these ridges.

Internal acoustic meatus.—The upper border of the meatus (fig. 17) is formed by the inner edge of the suprafacial commissure and a posterior continuation of this salience, while the lower border, lying considerably nearer the median plane, represents the upper edge of the medial wall of the cochlea. Both of these borders are sharply marked, while the anterior and posterior, joining them, are more rounded. From the anterior border is continued backward and downward the beginning of the spiral septum; the free edge of this septum may be followed forward and inward as it curves sharply to meet the inner border of the meatus, thus outlining the anterior end of the inferior acoustic foramen. The intermediate portion of the posterior meatal border is formed by the wall of the elliptic recess, which, as we have noted, narrows and descends into the depths of the meatus to join the upper part of the spiral septum and thus to form the transverse crest.

The superior acoustic foramen, opening into the elliptic recess, is situated lateral to the upper part of the crest, and looks directly forward, so that it can not well be seen in figure 17. The position is indicated by an arrow in figure 15. It represents the future superior vestibular area. Lying medial to the crest, and delimited medially by the sharp inferior border of the meatus, is the elongated inferior acoustic foramen. This is not shown well in figure 17, since it looks directly upward, but figure 15 discloses its medial wall. When viewed directly from above it is seen that the spiral septum approaches the ridge upon the medial cochlear wall (already mentioned) and thus the foramen is incompletely divided. In *Ia* there was a narrow cartilaginous union here. The anterior portion, slightly the larger, is the site of the future central canal and spiral foraminous tract; the posterior portion represents the inferior vestibular area and the foramen singulare. The latter opens into the cartilaginous canalis singularis (fig. 18) carrying the branch of the vestibular nerve to the ampulla of the posterior canal, and in this respect resembles the condition in *Ia* and also in the rabbit (Voit).

Thus we have, as in the mature bone, a space above the transverse crest, representing the fossula superior, containing the passageways for the facial nerve and the superior division of the vestibular nerve, and a space below the crest, representing the fossula inferior, for the inferior division of the vestibular nerve.

The facial nerve is shown in the internal acoustic meatus in figures 15 and 16. It passes almost directly outward, and a little downward, and lies in front of the vestibular ganglion. The genicular ganglion is placed a little beyond the lateral edge of the suprafacial commissure.

The *vestibular ganglion* is crescent-like in form. Its outer end—a thin point projecting laterally and forward—underlies the commissure, but does not reach as far as its outer border. It lies in front of the superior acoustic foramen. The upper extremity is tucked under the inner margin of the meatus. The ganglion narrows as it curves downward and backward, presenting a medial convexity, and terminates deep in the caudal end of the inferior acoustic meatus.

Massa angularis.—The angular mass, already referred to, is seen from the front in figure 24 and from below in figure 25. In figure 15 it appears from within, in its relation to the canal spaces and to the remaining cartilage of the capsule. Its lateral surface, quite smooth, has been noted in figures 5, 6, and 20. Thus it extends from the medial to the lateral surface of the pars canalicularis.

In direct antero-posterior views it presents a triangular outline, the lateral surface forming the base, and the region of the fossa subarcuata representing the apex. The mass of cartilage projecting from the lower border, in figure 24, is the trabecula inclosed by the lateral semicircular canal. In figure 25 it is seen trimmed more closely to the angular mass proper. A similar, though somewhat less obvious, triangular outline appears when the mass is viewed from above, or from below, as in figure 25, all the borders being more rounded and the apex, still in the same locality, being less marked. Seen from within or from without, the mass is roughly quadrilateral. The relation of the semicircular canals to its anterior, medial, and lower aspects may be seen from the figures. The fossa subarcuata penetrates deeply into its interior and ends in a very slight dilatation. Underlying the space for the crus commune (fig. 15), there is a ridge which becomes sharpened posteriorly, where it joins the trabecula inclosed by the posterior canal space. The cartilage is lightly staining with scattered nuclei.

The space within the otic capsule is very large. Figures 15 and 23, of the lateral and medial walls, and figures 18 and 21, which show the external

form of the cavity, modeled as a solid, will be helpful in gaining a true concept of it.

Cochlear space.—The cochlear space begins at the foramen perilymphaticum and proceeds forward in the trough-like inclosure for the commencement of the first turn of the cochlear duct and spiral ganglion. Here it is partially overhung by the low ridge already described, which projects outward from the cranial wall of the cochlea and is walled in laterally by the medial part of the spiral septum and outer wall of the capsule behind this. Continued forward, upward, and finally backward and downward, the space is seen to suggest strongly the spiral character of the adult bone. The spiral cochlear duct contained in this space makes one and three-quarter turns, as seen in figures 19 and 22.

Vestibular space.—The vestibular portion of the space is very capacious. There is but little evidence of the sacculation which is present in the osseous condition. Just above the superior opening of the medial acoustic meatus (representing the future superior cribriform area) there is a faintly marked depression for the recessus utriculi of the utriculus, the representative of the future recessus ellipticus. Of the recessus sphericus, there is little evidence. The foramina which pierce the wall have been mentioned.

The *semicircular canal spaces* are all large and all show dilatations for the ampullæ. They bear the usual relationships to each other and to the vestibular cavity. The plane of the lateral canal is very nearly parallel with that of the basal plate. In its course it circles around a short trabecula (cut in fig. 25), which joins the angular mass with the floor of the capsule just behind and lateral to the fenestra vestibuli. The caudal non-ampullated end opens into the vestibule immediately in front of the trabecula inclosed by the posterior canal.

Membranous labyrinth.—But little need be said as to the membranous labyrinth (figs. 19 and 22), since its features are familiar. It approximates in form the mature condition. All the parts are represented. As in the adult, it occupies but a comparatively small volume of the space contained within the otic capsule. The cochlear duct does not show such a distinct projection of the apex of the coil from the base as in the adult condition. Lying within the coil is the spiral ganglion, which ends in a slight enlargement, just medial to the smallest coil of the duct; this enlargement shows a short spur, directed toward the medial portion of the middle meatus. The ganglion ends dorsally at the angle between the dorsalmost straight portion of the duct and its basal limb, and thus does not reach the vestibular cæcum.

The *ductus endolymphaticus* is long and slender and crosses the crus commune in a direction from above downward and forward. Its saccus is broad, flattened latero-medially, and of oval outline, and it lies medial to the transverse sinus. Its dorsal extremity is a very slender filamentous process containing an almost imperceptible lumen.

Stapes.—The stapes (figs. 31, 34, 44) is a ring of cartilage with a thick cellular perichondrium. A section of the ring, representing the future foot-plate, is in the membrane filling the vestibular window. The ends of this primitive foot-plate are indefinitely marked off from the fenestral margins; indeed, anteriorly there is a union by young cartilage with the anterior edge of the fenestra, shown as a blunt spur in figure 20. This primitive foot-plate presents a marked bowing inward and upward, and pushes in the membrane filling the oval window so that it bulges into the vestibular cavity (fig. 15).

The section of the ring opposite to the foot-plate is bent a little to form a rounded angle which articulates laterally with the crus longum of the incus. Here the tendon of the stapedius muscle is inserted (fig. 44). The stapes makes with the crus longum of the incus an angle of about 90°.

Incus.—In form the incus approximates the mature bone, presenting a body and two crura. The body is separated from the adjoining head of the malleus by membrane, but as yet there is no joint cavity. There is a well-marked notch on the cranial aspect of the body for the cog-tooth of the malleus. A distinct spur of cartilage, tipped with young cartilage, marks the cranio-lateral limit of the body and projects forward to the upper extremity of the malleus. The crus breve points backward, with a curve of its tip downward (fig. 6) to apply the medial surface of its extremity (fig. 31) to the wall of the otic capsule just lateral to and a little below the anterior limb of the lateral semicircular canal—the site of the future fossa incudis (figs. 16, 20). This connection is accomplished by young cartilage. In *Ia* it was membranous. This represents the sole connection of the otic capsule with the first visceral arch. The crus longum, underlying the facial nerve, points inward, downward, and a little backward. It is parallel with the handle of the malleus, but is considerably shorter than this. The representative of its future processus lenticularis is edged with young cartilage.

Malleus.—The head of the malleus (fig. 6) is

directly continuous with Meckel's cartilage, showing no evidence of the future separation. The neck is relatively longer than in the adult condition, and the manubrium is shorter. The lateral process (fig. 42) is a distinct tubercle; here the neck and manubrium join to form a gentle curvature, with concavity upward and inward. The chorda tympani lies just medial to this between the processes of the malleus and incus. The manubrium, from the processus lateralis to the tip, is just medial to the stratum cutaneum of the tympanic membrane. Medially it is inserted into a distinct bay upon the lateral aspect of the developing tympanic cavity (figs. 16 and 42), the representative of the stratum mucosum of the future tympanic membrane. The head shows a ridge, edged with young cartilage, directed upward and backward, which represents its spur or cog-tooth. This is loosely fitted into the notch upon the body of the incus. A small tubercle is directed forward from the anterior aspect of the head.

The anterior process, or processus gracilis of Folianus, is a slender spicule of very young bone; indeed, most of the substance is composed of osteoblasts with but little ground substance. It arises in the perichondrium of Meckel's cartilage, some distance below the head of the malleus and a little below the chorda tympani nerve (figs. 31 and 34). It is attached to this perichondrium on the caudo-medial aspect of the malleus, and the spicule projects from this upward and a little backward and outward, slightly approaching the chorda, to end freely a little in front of the neck of the malleus. A connection, however, is made through connective tissue and the malleus shows at this point a small amount of young cartilage which indicates the future fusion of the process to the neck. This bone, homologous with the goniale of lower forms (Gaupp), was somewhat farther developed in *Ia*.

AUDITORY TUBE AND TYMPANIC CAVITY.

The auditory tube comes off from the pharynx just behind the level of the developing medial pterygoid plate and follows a course outward, upward, and slightly backward. It is much flattened from above downward and outward and there is a very gentle curvature with concavity downward and outward. At a point about half way between the pharyngeal opening and the caudalmost extremity of the tympanic cavity the expansion for the latter begins to appear, and the outer end of this epithelial evagination is very broad and flat. When the entire structure is looked at in frank view the medial border shows a much stronger inward concavity than the lateral.

The tympanic cavity presents practically no lumen, the walls being approximated. It lies quite close to the promontory of the cochlea. The medial aspect presents a shallow concavity and the lateral aspect a corresponding convexity, which, however, is even less marked. The upper area is specially modeled, presenting a depression (figs. 16 and 42)—representing the stratum mucosum of the future membrana tympani—for the manubrium of the malleus. From the superior border of this depression there are tongue-like prolongations which partially embrace the manubrium, as shown in figure 42. A little medial to this upper edge is found the end of the crus longum of the incus (fig. 16). There is a small convexity upon the medial aspect corresponding to the depression for the malleus (fig. 44).

TYPE OF CARTILAGE.

The type of cartilage of the otic region is almost all mature. In the angular mass it is pale-staining with sparse nuclei. The upper portion of the cochlea shows a younger type of cartilage than the remainder of the capsule. Edges of young cartilage are found bordering the endolymphatic and vestibular foramina, the spiral septum and the small ridge upon the opposite wall, the outer edge of the suprafacial commissure, and the joint surfaces of malleus and incus; and, upon the malleus, the lateral process, the tip of the manubrium, and the site of future union with the goniale. There are no ossification centers.

ORBITO-TEMPORAL REGION.

The orbito-temporal or sphenoidal region contains a central unpaired mass, representing the body of the sphenoid, and three lateral paired masses representing the two wings and the medial pterygoid plates.

The basisphenoid has been described with the basal plate. The anterior end of its connection with the cochlea juts out toward the side (fig. 10) and here forms part of the posterior boundary of the carotid foramen. This projection represents the posterior petrosal process of the mature bone.

The dorsum sellæ, less developed than that of *Ia*, is directly continuous with the basisphenoid and is slightly concave anteriorly. It terminates in two lateral, somewhat divergent and blunt tips, the developing posterior clinoid processes. The upper

edge is of a somewhat younger type of cartilage than that of the lower portion of the plate.

In front of the dorsum sellæ the body of the sphenoid, which here forms the floor of the hypophyseal fossa, is wide and thin and slightly hollowed, but unperforated. Low upon the lateral border appears the thin uninterrupted cartilaginous union with the alar process (fig. 10). Joining the root of this process with the posterior petrosal process is a narrow ridge, edged with young cartilage, forming the lateral border of the carotid foramen.

In front of this thin floor the cartilage narrows transversely and broadens dorso-ventrally as it passes forward. The upper surface presents four distinct elevations in front of the dorsum sellæ and, including the hypophyseal fossa, four depressions or notches between these. The anterior wall of the hypophyseal fossa is slightly hollowed and is surmounted by the tuberculum sellæ. To its extremities are attached the stout metoptic roots of the lesser sphenoidal wing (figs. 1, 10). The tuberculum sellæ is not so wide as in *Ia*, nor is there a middle clinoid process present, as there was on the right side in *Ia*.

In front of the tuberculum sellæ the cartilage, now known as the lamina hypochiasmatica, narrows rapidly from side to side and descends into the sulcus chiasmatis. The upper surface is slightly convex. To the lateral margins are attached, just in front of the caudal roots of the lesser wing (with which their bases are continuous), the alæ hypochiasmaticæ.

ALA HYPOCHIASMATICA.

These projections seem to be homologous with the orbital processes of Terry, in the cat. They are thin, wing-like structures of young cartilage with rounded borders of precartilage which project outward and a little downward into the optic foramina. Thus each forms with the anterior margin of the posterior root, a notch opening outward into the optic foramen. Anteriorly each wing is separated from the posterior end of the prechiasmatic commissure by another notch—also an extension of the optic foramen.

The ala hypochiasmatica of No. 886 is less developed than that of *Ia*. In relation to the orbital wing it is a very insignificant object in both stages and it is therefore of interest that Kernan (1916) has reported this structure in his 20 mm. human embryo as relatively very large, when compared with the lateral part of the orbital wing. He describes it as a free cylinder of cartilage in the position, apparently, of the future posterior root of the orbital wing, to which he considers it to belong. Lewis, in the 21-mm. stage, describes the same structure as the "basal part" of the orbital wing. Here, too, it is relatively very large when compared with the lateral part of the wing. Lewis thinks that the basal part "must ultimately become incorporated into the body of the sphenoid." From a consideration of these different stages it would seem that the ala hypochiasmatica is very precocious in its development, early attaining a prominence which is not maintained in later life. It is possible that the ala hypochiasmatica, as described by me in No. 886 and *Ia*, does not represent all of the structure described by Kernan and Lewis. It would be of interest to ascertain how far the development of this structure is concerned with the attachment of the muscles of the orbit. Fawcett (1919) finds, in the chondrocranium of the bat, all the ocular muscles except the two obliques attached to the hypochiasmatic wing, and Lewis (1920) reports a similar finding in his human embryo. Voit finds an independent ossification center in the ala in the rabbit chondrocranium.

PRECHIASMATIC COMMISSURES AND FORAMINA.

Connected with the edges of the hypochiasmatic plate (figs. 1, 10), lateral to the sulcus chiasmatis and a little in front of the hypochiasmatic wings, are the caudalmost extremities of the prechiasmatic commissures, slender strands of precartilage with extremities of young cartilage, which extend forward to the attenuated anterior roots of the lesser wings of the sphenoid. They are almost negligible when compared with those of *Ia* and are evidently just developing.

These commissures cut off the small prechiasmatic foramina from the optic foramina. Fawcett (1918, p. 425), on the basis of his researches with the chondrocranium of Weddell's seal, ferret, and cat, prefers to think of the prechiasmatic foramen as an isolated part of the orbito-nasal fissure rather than as a cut-off part of the optic foramen. These foramina are oval in shape, with long axes directed caudocranially, with a slight deflection of the caudal extremities laterally. Underlying them are the upper extremities of the ectethmoids. They were present in *Ia*, being a little farther apart and relatively smaller. They are also present in the Hertwig model.

In my former article I stated (p. 390), that the prechiasmatic foramina are not to be found in the osseous condition. This statement should be revised, for, although in some skulls they are not to be found, they may persist in others. I have

found them present in the sphenoid of a young adult in the osteological collection (No. 101) of the Johns Hopkins Medical School. The skulls in which they were absent were apparently from old individuals.

INTERORBITAL SEPTUM.

The part of the central stem forming the narrow medial delimitation of the orbital cavities is known as the interorbital septum. Its anterior end lies above the level of the posterior extremity of the ectethmoid. It is much reduced in area in the human subject as compared with lower forms. It extends forward slightly beyond the limbus sphenoidalis, coming to an end at the point where the attachments of the ectethmoid reach to the upper margin of the septum.

Anterior to the sulcus chiasmatis the upper border of the interorbital septum passes forward and slightly upward to reach a distinct eminence, the limbus sphenoidalis. Its upper narrow extremity is prolonged laterally into thin ridges, to which, at bilaterally opposite points, are attached the small preoptic roots of young cartilage. It is to the posterior aspect of these roots that the anterior extremities of the prechiasmatic commissures are attached.

In front of the limbus sphenoidalis is a much smaller notch, followed by a low but distinct eminence, capped by young cartilage. Somewhere in this region is the position of the future sphenoidal spine, although there is nothing here in the condition of the cartilage indicative of the future separation of the sphenoidal and ethmoidal elements of the septum. This ridge presents, on either side, very slender connections with the roof of the ectethmoid, and directly behind this are two other junctions with the same structure. These attachments, of which there are thus three on either side, are separated by minute foramina and lie in almost a straight line (fig. 10). The caudalmost junction is below the limbus. They spring from a ridge of cartilage which projects laterally from the septum. The anterior end of this is level with the upper edge of the septum, but the posterior end is some distance below the limbus, as figure 10 shows.

In front of the eminence just described the upper border passes forward upon the nasal septum, descending into a distinct notch and rising upon the very prominent crista galli, to fall away finally upon the anterior border of the mesethmoid.

The lower border of the body of the sphenoid, or more accurately here, the presphenoid, after leaving the distinct notch beneath the floor of the hypophyseal fossa, follows an almost direct course downward and forward, as figure 10 shows. Somewhere in the vicinity of the dorsal extremity of the vomer we have to place the ventral limit of the presphenoid, though, as with the upper margin of the plate, there is no trace of any delimitation.

PROCESSUS ALARIS.

The temporal wing proper is supported by the processus alaris (figs. 1, 2, 15), a short, rather plate-like rod of cartilage, flattened from above downward, which projects laterally at right angles to the body of the sphenoid, with but a very slight inclination downward. Its medial extremity, of which the cut edge appears in figure 15, is thin, and is attached to the lower surface of the body of the sphenoid, as shown in figure 10. The lower surface shows a slight concavity from before backward, and the upper surface a corresponding convexity. There is upon the upper surface, too, a slight side-to-side concavity, and in this the internal carotid artery lies (fig. 15). It is the representative of the future sulcus caroticus. The sulcus is bounded laterally by a raised, knob-like eminence, which marks the latero-dorso-caudal extremity of the alar process, and from this is directed, backward and slightly upward and outward, the alicochlear commissure (fig. 6), a well-marked spur of cartilage. This, it will be remembered, is confluent by its slender caudal tip with the cochlea just below the cranial pole. From its relation to the internal carotid artery and to the sphenoidal fundament, it is indoubtedly the primitive lingula which, in the mature skull, is formed of bone and projects backward into the foramen lacerum, often being quite elongated. Although the slender junction of this commissure with the cochlea is of young cartilage, the knob-like eminence from which it springs shows slight changes in the cartilage premonitory of ossification. This region represents the sphenotic center. Fawcett (1910) describes this independent center in a 110-mm. human embryo and remarks:

"Its large size is somewhat surprising, if it form only that part commonly in our text-books called lingula. From its posterior end a pointed cartilaginous process is seen to pass backwards towards the auditory capsule."

The alar process, thus, is for the most part included in the body of the mature bone rather than in the greater wing. Its outer extremity, with the continuation into the alicochlear com-

missure, forms the lingula. Lewis (1920) thinks it probable that the alar process is largely incorporated into the body of the mature bone and brings forward several lines of evidence favoring this view, including the relations of the carotid artery and the nerve of the pterygoid canal to the process, and also some interesting craniometric findings. He agrees in the general view that the alar process also forms the lingula, but does not mention the alicochlear commissure or alicochlear process.

ALICOCHLEAR COMMISSURE.

The alicochlear commissure is 20 micra in length and conical in form, The lateral diameter of its base is slightly greater than the dorso-ventral. With the projecting cochlear pole it forms the lateral border of the carotid foramen. Jacoby found it in the 30 mm. stage, but Levi did not describe it. Fawcett (1910*a*, p. 216) found that in a 30-mm. embryo "the processus alaris sends backwards a pointed process which comes into contact with the auditory capsule." He added that "this condition is even visible in the 110 mm. stage." It is not clear whether there were actual cartilaginous unions with the otic capsule. Kernan found, in the 20 mm. stage, an alicochlear process which came into contact with the cochlea but did not unite with it. He considers it to be only temporary in man. In the corresponding region of *Ia* there was a projection backward toward the cochlea, but no actual cartilaginous union.

CAROTID FORAMEN.

The carotid foramen is irregularly rounded in outline (figs. 1, 2) with a narrow lateral diverticulum between the alicochlear commissure and the cranial border of the cochlea. Views of its medial and lateral walls, which have been described, are seen in figures 10 and 15 respectively. The internal carotid artery occupies only this outer angle. It passes in an oblique direction from below upward, forward, and slightly inward, close to the alicochlear commissure, to overlie the alar process in the region of the future cavernous sinus. Its course beneath the otic capsule has already been noted. There is no indication of the canal through which the artery later passes in the osseus skull and it is evident that the future development of the region at the cranial end of the cochlea must be characterized by considerable extension, there being developed between the carotid foramen and the membranous cochlea a lengthy piece of bone. In the mature skull, too, the carotid foramen is merely the inner end of the foramen lacerum, since the backwardly projecting lingula does not reach to the petrous portion of the temporal bone, as a rule.

TEMPORAL WING.

The connection of the temporal wing with the alar process does not take place directly from the outer extremity of the latter, but rather from an area at the outer end of its ventral surface. This is somewhat triangular (fig. 16) with the apex directed forward and inward, and is gently convex. It is directed downward, forward, and slightly outward. The reverse side, upon the dorsal surface of the wing, appears in figures 26 and 27, its inner boundary being separated from the adjoining surface by a low ridge. In the sections where this division has been made, a very thin strip of young cartilage may be seen, which separates the two portions of the wing.

If the alar process and wing be looked at from below, there is seen a sharp notch where they join, but when the separation of the parts is effected as outlined above it is evident that this notch belongs to the alar process. In it is the nerve of the pterygoid canal, lying just above and a little medial to the caudo-medial angle of the wing (fig. 26). It is in this region that the future pterygoid canal will be formed. This notch is continued around upon the caudal aspect of the wing, and may be seen from the side, as in figure 6, between the lateral extremity of the processus alaris (above), and the caudo-medial angle of the ala (below).

The temporal wing, resembling that of *Ia* quite closely, is a rhomboidal block of cartilage which lies with its long axis directed forward, outward, and upward. It hangs altogether below the level of the alar process. Perforating the block from above, downward, and forward, is the conspicuous foramen rotundum (figs. 2, 26, 27), situated nearer the medial than the lateral border and nearer the anterior than the posterior end. The antero-lateral salient portion may be recognized as the ascending lamina, while the pterygoid process projects farthest ventrally and is just lateral to the medial pterygoid plate (fig. 29). The two surfaces may be described as dorsal and ventral, though they look a little posteriorly and anteriorly respectively. The dorsal surface (fig. 1) is somewhat convex from side to side and from before backward, and presents a sharp upward edge of young cartilage which borders the foramen rotundum laterally. In front of the foramen there is a narrow and

shallow transverse concavity, while medial to the foramen is a narrow isthmus of young cartilage which joins the thicker anterior and posterior parts (figs. 11, 26, 27). This isthmus is by far the most slender part of the wing and seems to be the last part of the foraminal border to chondrify; for in earlier stages the foramen is incomplete here, the greater wing appearing as a hook with its tip stretching medially, forming the notch which represents the foramen (Lewis et al.). In *Ia* it was stouter, though still of comparatively young cartilage. Behind the foramen is the stoutest portion of the wing. Its upper surface slopes downward and backward, its medial region, underlying the alicochlear commissure, presenting a low ridge, directed principally backward, which is edged with young cartilage. This ridge terminates caudally in the caudo-medial angle of the wing, some distance above the level of the pterygoid process.

The ventral surface, which also looks forward and inward (figs. 2, 3), shows the rounded lateral and superior portions of the foraminal border. The slender medial border is also seen. Just below the foramen is a rounded forward projection from the pterygoid process, which passes backward to be lost at the caudo-lateral angle of the mass. A shallow groove is medial to this ridge. It extends from the foramen to the caudal edge of the wing. Medial to this is a rounded area which curves upward to the mesial border.

The anterior boundary is quite sharply marked; the lateral is distinct in front, becomes blunt and rounded behind, and shows a shallow outward concavity. The posterior is best marked medially, where is terminates in the blunt point already mentioned. The medial border is thin in front (figs. 11, 27); from the slender foraminal portion of this the boundary is continuous, medially, with the thin cranial edge of the processus alaris (fig. 11), there being here a narrow edging of young cartilage. The caudal portion of the medial boundary of the wing is lost in the connection with the alar process, except posteriorly, where it runs over upon the caudo-medial angle.

Evidences of beginning ossification.—Particularly in the region of the pterygoid process, and lateral to and below the foramen rotundum (see Fawcett, 1910*a*, fig. 13), but extending out from this, especially forward, the cartilage shows evidence of preparation for ossification (figs. 11, 26, 27, and 29). It involves practically the entire outer half of the wing and extends inward to about the middle of the mass lying in front of the foramen rotundum. The cranial half, however, is not so strongly marked as the caudal and, as has been noted, the upper edge of the lateral margin of the foramen is edged with precartilage. Below this ridge the lateral wall of the foramen shows preossification changes.

The *semilunar ganglion* lies above the greater wing, though separated therefrom by an interval equal to its own thickness. Its posterior third is applied quite closely to the antero-lateral aspect of the cochlea (figs. 15, 16). Its root and branches are all well seen in these figures. The root pierces the dura just above the level of the ganglion. The ophthalmic division, much the smallest, passes forward through the wide superior orbital fissure, seen in figure 1. The maxillary division traverses the foramen rotundum (figs. 26, 27, 29) and gives off its sphenopalatine branches and twigs to the sphenopalatine ganglion (fig. 27), while the remainder of the fibers pass downward and forward as the infraorbital nerve. The mandibular division courses downward and a little outward and backward, as seen in figure 16. As yet there is no foramen ovale.

In figure 16 the course of the great superficial petrosal nerve may be followed as it passes forward close to the outer surface of the cochlea just behind the mass of the semilunar ganglion. At the cranial end of the cochlea it comes to underlie the alicochlear commissure and the internal carotid artery. It then turns more directly forward, diverging from the artery, which is now separated from it by the alar process. In making this turn it passes above the caudomedial angle of the temporal wing (figs. 26 and 27) and comes, as the nerve of the pterygoid canal, to occupy the notch between the process and the wing proper. Traced forward from this, it is seen entering the sphenopalatine ganglion, as the figures show.

The *medial pterygoid plate* (figs. 26, 29) is a thin strip of membrane bone which is directly continuous below and behind with the rounded parasphenoidal cartilage, representing the hamulus. The bone and cartilage form a right angle (figs. 26, 28). The hamulus alone is seen in figures 2, 3, and 6. The cartilage and bone seem to be quite intimately joined together, and there is no definite boundary between them—in fact, the appearance of the cartilage suggests that it is in process of calcification preparatory to ossification. The lower end of the anterior border of the plate resembles cartilage. In the description of *Ia* it was noted that this cartilage differs in histological appearance from that of the chondrocranium proper. The pointed upper end of the bony plate lies but a short distance below the caudomedial

angle of the greater wing. It is inclosed in condensed mesenchyme, which is continuous with that enveloping the palate bone, as seen in figures 28, 30, and 41.

ORBITAL WING.

The orbital or lesser wings of the sphenoid are seen from above, below, in front, and from the side in figures 1, 2, 3, and 5, respectively. The two wings make with one another an angle of 131°, measuring from the upturned tips of the dorsolateral processes and limbus sphenoidalis. The corresponding angle was measured in four adult skulls and found to range from 147.5° to 154.5°, the average being 150°.

The wings have been described by many authors as sickle-shaped plates of cartilage joined medially to the presphenoid and anteriorly continued as the spheno-ethmoidal cartilage. It is convenient to divide each wing into four parts: the body, the dorso-lateral process, and the two roots. The body is thin and almost plane anteriorly, while posteriorly it is thicker and narrower. It is this latter portion of the wing which is the first to develop, according to the accounts of earlier stages, as that of Lewis (1920), where the shape is also that of a sickle, but with the concavity of the blade directed inward to form the boundary of the optic foramen. The anterior root, in No. 460, has not joined with the main stem and in No. 886 it has evidently just joined, for it is very slender and of young cartilage. Lateral to the optic foramina, the upper surface of the body shows a gentle concavity upward and inward (fig. 1) and below this region there is a corresponding convexity downward. When seen from the side (figs. 5, 6) the combined lesser wings and spheno-ethmoidal cartilages present a distinct concavity downward, this plate of cartilage forming part of the roof of the orbit. The anterior border of the body forms the caudal boundary of the orbito-nasal fissure—a triangular cleft which partially separates the wing from the spheno-ethmoidal cartilage. The lateral border of the two plates, beginning in front at the spheno-ethmoidal commissure, describes a compound curve, the edge of the spheno-ethmoidal cartilage showing a strong outward convexity, which is followed by a gentle outward concavity as the line passes over upon the dorsolateral process. Just beyond this border is the posteromedial edge of the frontal bone.

The posterior border, beginning at the inner extremity of the superior orbital fissure, also describes a compound curve, the first portion of which is very sharply convex backward, where it passes around the pointed anterior clinoid process; there is next a long sweeping curve with concavity backward, the first portion of which shows a thickened margin.

The dorsolateral process is an almost straight cylindrical rod pointed backward, outward, and upward, and is somewhat thicker than the portion of the wing from which it springs.

The posterior root of the lesser wing is a short, stout rod of cartilage which springs laterally from the tuberculum sellæ (fig. 10). Each root shows a rather wide, shallow groove upon the dorsal surface, that on the right side being slightly better marked. A narrower groove is found upon the ventral surface of each root, which is continued forward as the groove separating the ala hypochiasmatica from the medial stem. From the anterior aspect of the junction of root and presphenoid, the ala hypochiasmatica is continued forward. The anterior clinoid processes are rather blunt and tipped with young cartilage.

Lewis (1920, p. 309) surmises that there are separate centers of chondrification for the two parts of the orbital wing, the basal and the lateral—a point which was advanced by Kernan (1916), who identified the "basal part" as the ala hypochiasmatica. Lewis thinks that the basal part is later incorporated into the sphenoidal body.

The anterior roots of the ala orbitalis, formed of young cartilage, are very slender. In *Ia* they were very much wider and stouter.

Optic foramen.—But little remains to be said as to the optic foramen. The lateral border, which may be considered to be formed by the margins of the body and the two roots, is semicircular in outline and lies at a higher level than the medial, which is made anteriorly by the prechiasmatic commissure and posteriorly by the outer margin of the hypochiasmatic lamina, bearing the salient hypochiasmatic wings.

The triangular *spheno-ethmoidal cartilage* (figs. 1, 5, 6) is confluent caudally with the lesser wing of the sphenoid. Its cranio-ventral angle is connected with the projecting upper edge of the ectethmoid by a narrow bridge, the spheno-ethmoidal commissure, and behind this the connection with the ectethmoid along the inner border of the cartilage is accomplished by several cartilaginous strands. This connection is very imperfect caudally. Most of the caudal border—that medial to the connection with the orbito-sphenoid—is free, forming the cranial limit of the orbitonasal fissure. The medial part of this border is irregular, and marked by projections of young cartilage into the fissure. The part of the

plate in front of this border is perforated by several small holes. The spheno-ethmoidal cartilage shares with the orbitosphenoid in the formation of the cartilaginous roof of the orbit and the floor of the anterior cranial fossa. It is almost plane, showing but a suggestion of a downward concavity.

The *orbitonasal fissure* (figs. 1, 5, 6) lies between the inner portions of the orbitosphenoid and the spheno-ethmoidal cartilage. The cranial border, irregular and broken by projections of young cartilage from the spheno-ethmoidal cartilage, lies at a lower level than the caudal, which it meets at a sharp angle, marking the lateral limit of the fissure. The caudal border, rather blunter, shows a gentle forward bend near the point where it passes over upon the slender anterior root of the orbital wing. The medial border may be followed forward along the laterally projecting edge of the sphenoidal limbus; from the cranial extremity of this it crosses over the surface of the tectum nasi to join the cranial boundary. The medial portion of the fissure will doubtless become somewhat cut off by reason of the enlargement of the connection of the lesser wing with the main stem. Laterally the fissure overlies the orbital cavity, and medially its plane is above an inward extension of this cavity overlying the tectum nasi.

TYPE OF CARTILAGE.

The material of the orbitotemporal region is for the most part mature cartilage. In the alar process and greater wing, changes in the cartilage have been noted which indicate the position of the future centers of ossification here. There is rather more precartilage and young cartilage than in the posterior regions of the chondrocranium. The details have already been mentioned.

ETHMOIDAL REGION.

The ethmoidal region contains the mesethmoid and paraseptal cartilages, the ectethmoids and related cartilages. It is noticeably less developed than that of *Ia*.

MESETHMOID.

The shape of the mesethmoid can be best ascertained from figure 10. Three slender connections with the ectethmoid are noted upon its upper margin, while anteriorly there is a more elongated union. The slightly thickened crista galli is conspicuous above, and the upper border of the septum for some distance behind this separates the fenestræ cribrosæ (fig. 1). There is a small union on either side with the paraseptal cartilage. This was present on the right side only in *Ia*. The mesethmoid is thinner than that of *Ia* and does not possess such a thick lower border. There is no indication of a superior paraseptal cartilage, as in *Ia*.

The *ventrolateral processes* are rudimentary, being low ridges of very young cartilage extending out slightly from the lower thickened border of the septum (figs. 12, 13) just in front of the paraseptal cartilages. For some distance behind these processes the septal margin between the paraseptal cartilages is of a very young type of cartilage, covered with precartilage.

Mucous membrane of septum.—Models have been made of the mucous membrane covering the medial (fig. 37) and lateral wall (fig. 38) of the right nasal cavity. The mucous membrane of the septum, shown in figure 37 (which should be compared with figure 10 to get the relation of the membrane to the septum) shows a surface almost plane. There is an exceedingly low, indistinct, and wide elevation near its middle, which runs almost parallel with the upper border of the septum. Very low hollows border this ridge.

The *vomeronasal* or *Jacobson's organ* appears upon either side of the nasal septum. In figure 37 the mucous membrane of the right side has been cut away to expose the organ. It consists of a dorsal enlarged portion, of somewhat fusiform shape, which terminates dorsocaudally in a fine point. From the lower end a fine tube leads downward and outward to empty into the lower portion of the nasal cavity. The main mass of the organ is a congregation of more or less uniform cells which suggests a coiled tube, but the lumen is very indistinct and the walls are made up of several layers of cells. The septum does not show a distinct concavity for the organ, as in *Ia*.

The *paraseptal* or *Jacobson's cartilages* (figs. 12 and 13) show a much younger type of material and are more rudimentary in form than those of *Ia*, but the two main masses, medial and lateral, may be recognized.

The lateral mass is relatively small. It is roughly of triangular shape, the apex being directed forward, and being separated from the fundament of the ventro-lateral process by a narrow cleft. This end of the plate is of precartilage. The base, of young cartilage, is con-

tinuous medially with the medial mass, near the anterior end. The lateral edge is situated a little higher than the medial, making the lower surface look somewhat outward as well as downward.

The medial mass is a vertical plate of very young cartilage, edged with precartilage, which shows but little of the differentiation that characterized its condition in *Ia*. In shape, instead of being roughly quadrilateral, as in *Ia*, it is like a right-angled triangle, the projecting right angle being situated caudoventrally. The horizontal base is notched. The posterior side, longer than the base, is vertical in position and is bowed outward a little about its middle. It terminates below in a very slight enlargement. Its outlines are indefinite, merging into the surrounding mesenchyme, and it is of precartilage. The vomer is some distance behind it and is situated closer to the midline. The hypotenuse runs practically parallel with the lower edge of the septum and is but little above this, so that the portion of the septum lying between these plates is very small. The anterior angle is continuous with the septum (fig. 10) and with the fundament of the ventrolateral process. The latter connection was not present in *Ia*. This end probably represents the cranioventral process of *Ia*. A connection with the lateral mass, just behind this region, seems to represent the position of the ventral process of *Ia*. The surfaces of the plates look almost directly outward and inward. They are separated by an interval but little wider than the lower edge of the septum and lie practically parallel with one another.

These cartilages, being of such young material, are evidently developing rapidly and would doubtless soon have become differentiated as in *Ia*. They are somewhat difficult to outline, for they lie in a mesenchyme of which the parts, as modeled, are often but the most condensed portions. No connection could be made out between the lateral mass and the paraseptal process of the ectethmoid, which project toward one another and which are linked by a zone of loose tissue in *Ia*.

ECTETHMOID.

The ectethmoid is a thin crumpled plate of cartilage which presents on the outer surface a number of eminences and depressions, which appear on the inner surface in reverse. In addition, the inner surface shows the developing conchæ. In the ectethmoid there are represented a roof, a lateral wall, and a floor, though the first and last are not extensive.

The roof occupies little more than half of the entire length of the ectethmoid and is bounded laterally by the attachment of the sphenoethmoidal cartilage. The border is completed anteriorly by the upper edge of the inturned ectethmoid which meets the crista galli; the boundary of the posterior part of the roof is indefinite, being marked by a low medially curving ridge, which ends somewhat behind the paraethmoidal process in the region of the limbus sphenoidalis.

Most of the roof is incomplete, showing anteriorly the wide fenestra cribrosa (fig. 1). Its margin presents no cribro-ethmoidal process, as in *Ia*. Posteriorly the roof is joined to the nasal septum, and is of young cartilage. Some four or five small holes appear in this evidently newly formed part. Behind this area the roof can hardly be said to exist, being represented only by the sharply rounded upper edge of the antorbital plane. The posterior part of it underlies the preoptic root of the lesser wing.

The side wall is irregular in formation and anteriorly turns inward to meet the nasal septum. Underlying the lesser wing and spheno-ethmoidal cartilage the upper border flares outward and thus gives rise to a shallow and wide groove below.

The posterior or orbital portion is flat and shows near its upper extremity the small para-ethmoidal process of young cartilage. This has a blunt, free posterior end. In *Ia* there was here a free nodule of cartilage, which lay very close to the wall.

The upper border is connected anteriorly with the nasal septum through the roof; posterior to this it turns sharply downward to terminate in a free and very thin edge which, however, is continued downward beside the nasal septum as a membrane. The wedge-shaped presphenoid lies between these downturned parts of the border, and so they are widely separated behind. They are separated from the presphenoid by the very narrow cupulo-septal fissure.

The posterior end of the antorbital plane is somewhat indented, but does not show such a sharp dorsal palatine notch as did *Ia*. The upper corner, though turned downward as a sharp point of young cartilage, does not turn forward to form a distinct posterior cupular process, as in *Ia*. The ventrocaudal corner shows a backwardly projecting point of young cartilage.

The lower part of the plate turns in sharply to form the inferior concha. At the anterior end of the angle thus formed is the posterior nasal prominence, bearing the posterior maxillary process, formed of young cartilage.

The *posterior maxillary process* is considerably smaller than in *Ia*, and between it and the pos-

terior prominence there is a distinct cleft. Projecting forward from it is a hardly distinguishable osseous fragment—the beginning lacrimal bone. Immediately lateral is the hook-like *paranasal process* which shows a rather knobbed extremity, lying somewhat below the level of the lacrimal bone. The process projects downward and forward and its pedicle is slightly constricted. It is of a young type of cartilage. In *Ia*, it was disconnected from the wall. Medial to the process is the lacrimal duct (fig. 39).

Paranasal process.—The wall to which the paranasal process is attached is the widest part of the lower border of the antorbital plane. This ridge continues anteriorly, with an inward, upward, and forward curve, upon the posterior prominence. It corresponds to the deepest part of the middle meatus.

It is convenient here to examine the inferior concha, which forms the greatest part of the floor. It is inclined a little downward and shows a downward-looking, wide, shallow groove. This is of thin young cartilage. It is represented in the floor of the middle meatus by a low, rounded anteroposterior ridge. The free border of young cartilage is thickened and turned downward and is somewhat irregular. The caudoventral angle is rounded and the caudal border shows a backwardly projecting, short point, surmounted by a low vertical ridge of precartilage. Between this point and the ventrolateral angle of the plane there is a notch, the representative of the ventral palatine notch of *Ia*, which, however, is small when compared with the older stage. In it is found the anterior extremity of the palate bone (fig. 41). The concha gradually projects more steeply downward, as it is followed forward, and comes to an end some distance below the posterior prominence at the post-transverse incisure.

The anterior or nasal portion of the wall is more irregular than the posterior. Above appears the small superior prominence, which is not represented upon the internal surface by a concavity. A little below and behind it is the foramen epiphaniale. The middle prominence (called by Voit the supraconchal prominence or Sakterwulst) in less conspicuous than in *Ia*. Overlying it is a band of mesenchyme continuous with the frontal process of the maxilla. A corresponding but relatively less marked concavity appears upon the inner surface. Although there is a slight depression here in the mucous membrane, which is continuous caudodorsally with the cleft for the middle meatus, yet it is not so prominent as the eminence on the outer surface would lead us to expect. The inferior prominence, in front of the paraseptal process, is quite evident.

Cupular process.—The lower margin, at the front of the anterior naris, presents several projections. Anteriorly is the curious, slender, hook-like cupular process of precartilage (figs. 38, 39, 40, 41), which is an extension of what I termed the cupular cartilage. It extends for 700 micra anteroposteriorly. It at first curves sharply inward, coming to underlie the septum and also to lie quite close to its neighbor of the opposite side. It terminates in a sharp tip turned outward and backward. Only its stump was described in *Ia*. It shows a distinct outward concavity which embraces the epithelial plug of the anterior naris, as shown in figure 40. Rehmke (1913), according to Peter (1913), showed that this process later becomes cartilaginous and forms the medial crus of the greater alar cartilage, and that in doing so it fuses with what seems to correspond to the ventrolateral process. Rehmke's latest stage was 275 mm. sitting height (30–32 weeks).

Behind this process is a notch and the edge here is of very young cartilage. We then come upon the *superior alar process*, which projects downward from the lower edge of the inferior prominence. Behind this is the distinct *paraseptal process*, terminating in a point of precartilage which projects backward and upward. It forms the lower border of the *post-transverse incisure*. This incisure is made a foramen by the lower edge of the maxilla. Both the superior alar process and the paraseptal process are found in Rehmke's figures in Peter's Atlas. The former is included in the lateral crus of the greater alar cartilage, while the latter breaks up to form the lesser alar cartilages, as shown in Peter's figure 56d, from the 275 mm. stage.

The *interior of the ectethmoid* (figs. 41 and 43) presents all the young conchæ. There are three slender connections above with the nasal septum and anteriorly an elongated union with the septum appears. These all fit to the cut edges shown upon the septum in figure 10. Behind the posterior connection is seen the down-turned edge of young cartilage already mentioned. This was more advanced in *Ia*, where the appearance suggested a concha. Just below it, and above the posterior end of the superior concha, is a space which represents the spheno-ethmoidal recess. The *superior concha* is short, broad centrally, and low. It is of very young cartilage, with an envelopment of precartilage. The anterior extremity is rather narrow and turned downward. Underlying this concha is the shallow *superior meatus*.

The *middle concha* is well marked and widest in its central region. It overhangs the middle meatus and does not show a perforation, as it did in *Ia*. Posteriorly it does not pass over upon such a distinct ridge joining the posterior border of the inferior concha as in *Ia*, but there is a low ridge of young cartilage joining the two and development is evidently proceeding rapidly here.

The *middle meatus* is very capacious. Its floor, as already observed, is steeper in front than behind and it continues forward into the cavity formed by the middle prominence; beyond this the concavity leads downward into the anterior naris. The deepest part of the meatus is the posterior. About its middle is the process of the middle meatus, altogether of precartilage, which represents the isolated *cartilage of the middle meatus* of *Ia*, together with its pedicle of precartilage. It projects almost straight backward for 600 micra to end freely. It may represent the uncinate process. The connection with the wall is at the medial side of the posterior prominence. The cartilage of the wall is quite thick here and the part joined to this process is partially separated from that lying laterally by an upwardly-directed cleft.

The *inferior concha* needs no further description.

The *inferior meatus*, below the inferior concha, is seen in figure 41. The floor, in front, is formed by the paraseptal process, while behind this it is made by the developing premaxilla and maxilla. The paraseptal process comes almost into contact with the cranial end of the condensed mesenchyme of the premaxilla and the post-transverse incisure above it leads backward into the meatus. The end of the nasolacrimal duct is shown in figure 41. The inferior meatus is very narrow as compared with the middle.

Just in front of the paraseptal process is the concavity formed by the inferior prominence. This is quite evidently the wall of the future vestibule. Above the indentation for the middle prominence is a low ridge which probably represents the agger nasi. It is continued backward to the anterior end of the middle concha.

The *mucous membrane of the outer nasal wall* is shown in figure 38, which should be compared with figure 41 drawn from the same point of view. We note all the turbinate folds, covering their respective cartilaginous conchæ. The superior membranous concha is the shortest and least distinct and the two lower are very well marked. The superior meatus is short and shallow and the middle one is deeper and forms a very distinct cleft. The inferior meatus, longest of all, is quite shallow but projects into the meatus as a wide double layer of epithelial cells. This is shown from without in figure 39, where it is seen that the epithelium underlies practically the inner half of the inferior concha. In figure 41 the tip of the nasolacrimal duct, which is here somewhat expanded, is seen projecting into the space of the inferior meatus; its relation to the epithelium is seen in figure 39.

The caudal extremities of the meatuses show the familiar approximation where the nasal capsule narrows into the elongated nasopharyngeal canal, as shown in figure 38. This canal is supported laterally by the palate and internal pterygoid bones, in their common investment of mesenchyme (fig. 41).

NASOLACRIMAL DUCT SYSTEM.

The nasolacrimal duct system was modeled and a view of it is seen in figure 39. Commencing laterally, we note the two lacrimal ducts, terminating in the punctæ lacrimaliæ and opening into the somewhat dilated nasolacrimal sac. From this point the nasolacrimal duct runs directly backward for some distance, crossing above the maxilla and coming to lie between the lacrimal bone and the tip of the paranasal process. The duct does not lie in the depths of the notch which is made by this process and the posterior maxillary process. It now curves downward, inward, and backward, under the posterior maxillary process, terminating in an expansion (fig. 40) which is contiguous with the epithelial plate of the future inferior meatus (fig. 39). It has not yet broken through the epithelium. Throughout its extent no definite lumen can be recognized in this system, though the epithelial cells are arranged in ring-like formation with a potential lumen within.

The lower portion of the nasolacrimal duct in the human fetal skull is situated some distance posterior to that in the lower forms, as (to cite one of numerous examples) the chondrocranium of the cat (Terry), where the duct passes forward lateral to the anterior transverse lamina to empty into the nasal cavity in front of this lamina. The anterior transverse lamina is represented, in the human chondrocranium, by the paraseptal process and, if the position of this part of the duct were the same in homo as in the lower forms, it would pass forward around the paraseptal process to connect with the mucous membrane of the nose in front of this process. It would then empty into an extension of the anterior naris, as it does in the lower forms. Instead, it is situated far behind the

paraseptal process and its ostium is, accordingly, shifted backward from its earlier position. It would appear that the present position of this portion of the duct in man, far behind the paraseptal process, is consequent upon the disappearance of the cartilaginous connection between the paraseptal process and the nasal septum, which completes the anterior transverse lamina in the lower forms.

BASAL FISSURE.

The basal fissure (fig. 2),which is represented by a fenestra in most of the lower mammals, extends throughout the entire length of the ectethmoid, being continued forward as the incisura narina. Its lateral border has recently been outlined in the discussion of the lower border of the ectethmoid and its medial border follows the lower margin of the septum, being indented by the paranasal cartilages. The lateral and medial borders meet in front of the confluence of the ectethmoid with the septum. There is no such confluence behind, as we have seen, the caudal extremity of the ectethmoid being separated from the medially lying septum by the very narrow cupulo-septal fissure. This fissure runs forward to the caudalmost junction of the ectethmoid with the main stem of the chondrocranium. The basal fissure is interrupted by the inward projection of the alveolar process of the maxilla.

TYPE OF CARTILAGE.

Summarizing the type of cartilage in the ethmoidal region, we note that it is younger than that of the other regions of the chondrocranium. It has long been known that this region of the skull develops later than the others and accordingly it shows a rapid growth at this stage. In the region of the connections with the paraseptal cartilage, and with the upper portion of the ectethmoid and at the crista galli, the septum shows a young type of cartilage. In the ectethmoid, the roof and edges are, for the most part, of young cartilage, while the two upper conchæ and the posterior part of the inferior concha are lined with precartilage. Precartilage, too, forms the process of the middle meatus and tips the paraseptal and posterior maxillary processes.

CARTILAGINOUS BRANCHIAL-ARCH SKELETON.

MECKEL'S CARTILAGE.

Meckel's cartilage (figs. 1, 3, 5, 6, 31, 32, 34) is a long rod of mature cartilage continuous above with the head of the malleus and terminating below in a tip, turned forward and inward, which approaches very close to, but does not meet, its neighbor of the opposite side. The cartilage is of practically uniform thickness throughout, excepting at the angle near the tip, where it is flattened from above downward and forward, and widened. In front of this the cartilage rapidly narrows to a point. There is a constriction just below the head of the malleus. The two rods converge to form a wide angle (figs. 3, 4), in which lies the floor of the mouth. The shaft, when seen from the side, as in figure 6, has a practically straight course with a very faintly marked anterior concavity. When seen directly from the front there is, just above the angle, an inward bending, which makes a gentle concavity outward and downward here. Above this point the shaft appears straight from this point of view. Throughout almost its entire extent the cartilage (as shown in figures 5, 31, and 34), is covered by the mandible. At the lateral surface in the region of the ventral bend (fig. 32), there is a very close approximation of the cartilage to the mandible, and here the cartilage is undergoing change preliminary to ossification. The perichondrium here has become ossified. The modified cartilage cells extend far into the interior. The corresponding area, on the mandible, is seen in figs. 33 and 35. The relation of Meckel's cartilage to the slender goniale has been referred to.

STYLO-HYOID ARCH.

The *cartilage of Reichert*, representing the future styloid process, has been described with the otic capsule.

HYOID CARTILAGE.

The hyoid cartilage (figs. 5, 6, 45 46, 47) is roughly semicircular in form, resembling closely the mature bone. It consists of a body and paired lesser and greater cornua.

The body, cartilaginous, with an edging of young cartilage in regions adjoining the cornua, is oval in form when seen from the front, with a rounded cranioventral face. The caudal face presents a distinct transverse notch, as seen in the mid-sagittal section (fig. 46). Just above this the upper edge of the thyroid cartilage is confluent with the hyoid. At the site of connection with the lesser cornu the cartilage is somewhat pointed, but there is no direct cartilaginous connection here, a fine strand of membrane joining the two.

The greater wing, on the contrary, shows a direct continuity of its cartilage with that of the body.

The lesser cornua are two thin, fusiform bodies of young cartilage covered with precartilage, which are connected by fine strands of membrane with the body of the hyoid below and with the styloid process above.

The greater cornua, directly continuous with the body, spring backward to meet the superior cornua of the thyroid cartilage, there being a thin strip of membrane intervening. The anterior surface near the body, and the caudal tips, show precartilage.

THYROID CARTILAGE.

The thyroid cartilage, too, has acquired most of its adult form, but there are some outstanding differences. We note at once, from the figures, that its upper edge is closely associated with the body of the hyoid and histological examination reveals a continuity of the cartilage. Thus the hyo-thyroid membrane has not developed. The laminæ approach one another so as to form an even, rounded convexity ventrally and a corresponding concavity dorsally. Thus there is no evidence of the laryngeal prominence, the hyoid body even projecting farther forward than the thyroid cartilage, as figures 5, 6, and 46 show. There is no thyroid notch as in the adult; on the contrary, if the young cartilage is removed, there is a distinct notch on the lower border of the thyroid cartilage. This, however, is closed by a bridge of young cartilage, and above it there is a small foramen with precartilaginous borders, where chondrification is incomplete. The line of junction of the two laminæ, although cartilaginous, is edged with young cartilage. It shows, upon the inner or dorsal surface, just above the foramen mentioned, a low ridge (fig. 47). There is no evidence of an oblique line. An inferior thyroid tubercle is distinctly seen upon the lower border and also a low superior thyroid tubercle, projecting laterally from just below the upper border—rather closer to it than in the adult. The lateral surface presents a shallow concavity, running craniocaudally. The superior border makes a rather sharper angle with the superior cornu than is the case in the adult. The superior cornu is directly connected by cartilage to the lamina, the portion immediately above this connection being more slender than the terminal portion. The extremities lie caudomedial to the extremities of the greater cornua of the hyoid, but, although they are closely approximated (figs. 46, 47), they show no continuity, being separated by a thin sheet of membrane. It is to be noted that the superior cornu is continuous throughout, not interrupted by the lateral hyo-thyroid ligament, as in the adult. The triticeal cartilages are doubtless vestiges of this cornu. The upper extremity is covered with young cartilage.

The inferior cornu is much shorter than the superior, and rests upon the shoulders of the cricoid, as shown in figures 45 and 47, being separated by a thin sheet of membrane. The lower extremity is edged with young cartilage.

CRICOID CARTILAGE.

The cricoid cartilage displays its signet-ring form, the lamina, bearing the young arytenoids, lying between the inferior cornua of the thyroid cartilage. The lamina presents posteriorly a ridge in the midline, and from this the sides slope down to the vertical ridges, which join the positions of the future facets for the arytenoid and inferior cornu and divide the lamina from the arches. At the upper end of the posterior ridge is a small notch, almost completely filled with precartilage, which extends the superior opening of the cricoid here. To either side of this notch are the areas bearing the arytenoids. As figure 47 shows, there is a direct continuity of the precartilage investing the arytenoid with that covering the upper portion of the lamina. When, however, this investment is removed, as was done upon the right side in the models (fig. 47), it is seen that the arytenoid is separated from the lamina of the cricoid by a thin strip of membrane, representing the position of the future articular cavity. The part of the lamina bearing the arytenoid is raised into a tubercle, best seen from the side.

The upper border, passing forward upon the arch, shows in front of this tubercle a notch and (in front of the region for articulation with the inferior cornu) the lower border also shows a notch, the two serving to constrict the region between the lamina and the arch. In front of both arches there are tubercles, seen in side view upon the upper and lower borders. In front of the upper of these the border descends rapidly, the two lateral borders meeting to form a distinct notch in the midline, flanked by precartilaginous tubercles (fig. 45). In front of the notches upon the lower border the contour descends a little, but there is no notch upon the lower border, as seen from the front. The anterior section of the arch is by far its most slender part. Like the mature cartilage, the inferior opening is rounded, while the superior is oval, showing flattened sides.

ARYTENOID CARTILAGES.

The arytenoid cartilages are in a very undeveloped condition, being represented by paired masses of precartilage of rather indefinite outline, investing cores of cartilage of young type, which outwardly grade off into the surrounding tissue. The precartilaginous arytenoid, as I have modeled it, shows the surfaces and borders of the adult condition. The dorsal surface is slightly concave from before backwards, and even less so from side to side. In its ventral portion the cartilaginous core comes to the surface (fig. 47). This surface shows caudally a continuation of the precartilage over the surface of the cricoid lamina. The medial surface, smallest in area, presents a roughly triangular shape, with the apex cranial (fig. 46). The wider ventrolateral surface is somewhat hollowed out, as in the adult condition. The ventral border is convex downward, presenting a blunt processus vocalis; here the core of cartilage shows a corresponding projection (fig. 47). The dorsal border is sharply concave, while the lateral border shows an outward convexity with a distinct muscular process as the posterior extremity. The entire mass is, as already noted, fused with the upper portion of the cricoid lamina. The cartilage proper, though separated from the cricoid by membrane, is closely applied to it and is to be described as a cylinder having a general caudocranial direction, with a slight medial inclination. Upon its ventromedian aspect, rather nearer its cranial than its caudal end, is a spur of cartilage, the core of the vocal process, projecting downward and inward (fig. 47). The cavum laryngis is very narrow. There are no corniculate and cuneiform cartilages.

TRACHEAL RINGS.

The tracheal rings lie parallel with the basal plate and are of a very young type of cartilage. The upper three show fusions with one another. Each describes about half a circle. Only the upper four were modeled.

MEMBRANE BONES.

INTERPARIETAL.

The interparietal part of the future occipital bone is represented in No. 886 by two slender osseous spicules, lying one on either side of the processus ascendens (figs. 1, 4, 5), just above the upper border of the tectum posterius. The outer ends are somewhat medial to and below the projecting extremities of the parietal plates. Each half is slightly flattened antero-posteriorly and is invested by condensed mesenchyme, as seen in figure 14. This bone could not be modeled in *Ia* and is not included in the Hertwig model, so that comparisons with these specimens could not be made.

PARIETAL.

The parietal bone is a very fragile reticulum of osseous substance lying in a plane just lateral to that of the parietal plate. It is very small in comparison with the frontal bone. In outline it is roughly oval with the long axis dorsoventral. The bone is very slightly concave inwardly and lies in the membranous calvarium. Its caudal edge does not as yet overlap the cranial edge of the parietal plate, as it does in *Ia* and the later Hertwig model. The peripheral part is but little more delicate than the central. The texture of the bone was too tenuous for modeling, so that it had to be represented by profile reconstruction. The dorsal edge does not extend above the line joining the dorsal extremities of the parietal plate and frontal bone. In *Ia* the bone has grown enormously upward and in the Hertwig model this growth is even more marked, while the parietal plate has undergone reduction.

FRONTAL.

As in the case of the parietal, the outline of the right half of the frontal bone was worked out in plaster of paris and the details of structure were obtained by profile reconstructions in the sagittal and transverse planes. From the side (fig. 5) the bone appears as a roughly rounded plate bent into an outward convexity. The margins are serrated. Arching across this surface is a low but distinct ridge which separates the orbital from the frontal portions. This ridge is recognized as the representative of the supraorbital margin and probably the superciliary arch as well. It slants much more sharply backward than in the adult and extends from a projection of the bone in front, representing the medial angular process, to a point some distance behind the dorsocaudal angle of the bone. The caudal limit doubtless represents the position of the future zygomatic process. The zygomatic bone is separated from this point by a wide interval. This ridge marks the densest part of the bone and appears to be in the position of the original center of ossification, as I have pointed out (Macklin

1914, p. 416). The densest bone (surrounded by a dotted line in fig. 1) forms a crescentic plate of homogeneous osseous tissue, from which gradually narrowing spicules radiate to the periphery. The relation of the bone to the cerebral hemisphere is seen in figure 7.

The orbital portion of the bone forms an antero-lateral extension of the surface of the orbito-sphenoid and spheno-ethmoidal cartilages. Its lower surface is very slightly concave, being molded for the orbital cavity, and its upper surface shows a corresponding gentle convexity, where it forms the peripheral portion of the floor of the anterior cranial fossa. Its tissue, although reticular throughout, is rather denser than that of the frontal part above the region of the supra-orbital ridge. The inner margin of the plate lies but a short distance from the edge of the ala orbitalis and spheno-ethmoidal cartilage (fig. 1), though never in actual contact. In *Ia* this edge had grown under the cartilages mentioned.

It is apparent from a comparison of the mature bone with the present specimen that the spheno-ethmoidal cartilage must undergo resorption, the orbital plate growing backward and inward, to join the ethmoid and the lesser wing of the sphenoid. The result of this process is to form practically a right angle by the approximation of the margins articulating with the sphenoid and with the ethmoid. Already there is an indication of the beginning of such an angle in a spur of bone jutting inward and backward from the margin in question toward the spheno-ethmoidal cartilage.

The frontal portion forms by far the largest part of the bone and makes with the orbital portion a very obtuse angle. It presents a concavity looking inward and upward; in antero-posterior planes, however, this concavity does not appear, the bone in such sections being almost straight. Its margin describes rather more than half a circle. Upon the exterior no frontal tuberosities are yet apparent.

MAXILLA.

The maxilla is cancellous in structure. In it the main features of the bone as at term can be made out. The facial surface looks ventro-laterally—much more laterally than in the adult. It shows a gentle outward convexity and, although presenting roughenings and small holes, is nevertheless smooth in comparison with the medial or palatine surface, which is very rough on account of numerous bony excrescences, and is very slightly concave. The palatine surface is seen from within in figure 26, and from the front and a little above in figure 27, but neither of these figures gives a frank view of this surface. The facial surface is shown from below in figure 3 and from the side in figure 5, but here, again, its full area does not appear. If the bony processes are removed, these surfaces become very small. It is hardly possible to speak of the orbital and infratemporal surfaces, for the area which represents them embraces merely the groove for the infraorbital nerve and a very insignificant region behind this, which comes to an end at the caudal extremity of the alveolar process.

The palatine surface presents a very interesting condition. There is a distinct wide groove containing mesenchyme, which begins above just in front of the apex of the frontal process and traverses the bone to end below 0.2 mm. from the medial extremity. The lower end is wider than the upper and is straight, while the upper turns forward a little, as shown in figure 26. This groove represents the incisive suture, marking the line of division between the maxillary and premaxillary elements, and agrees in position with the figures of Felber (1919), who has recently investigated the development of the maxilla (see abstract by Schultz, 1920). The borders of the groove are sharply marked and conspicuous, the upper being the longer and reaching to the apex of the frontal process. These borders are somewhat nodular and at the lower end they project medially as processes, the posterior being the longer. Behind and lateral to this is the alveolus for the canine tooth, while medial to the lower end of the anterior border of the groove, in the territory of the premaxilla, are found the alveoli of the lateral and medial incisors (fig. 29). The floor of the groove is fairly smooth. Above, it is incomplete, there being a cleft, 0.1 mm. long, which extends through to the facial surface just behind the anterior border of the groove. A little behind this there is a much shorter fissure.

Felber has shown that during growth of the maxilla the premaxillary and maxillary elements preserve a certain independence. They arise from separate centers. The frontal process grows upward as two spicules, a maxillary and a premaxillary, separated by a cleft, and the union of the two elements occurs from the alveolar process upward. Thus it is that the elements unite last at the apex of the frontal process. Their fusion, too, is completed earlier on the facial than on the palatal aspect.

The body of the maxilla may be defined as all that is left after the processes are removed, and since the processes are the most prominent parts they will be described first.

The frontal process is thin and triangular in shape and lies in a hollow on the lateral aspect of the ectethmoid, between the region of the lacrimal bone above and the paraseptal process and adjoining cartilage below. With the basal plate horizontally placed, it projects almost directly forward and lies in a sheath of condensed mesenchyme which reaches forward around the developing nose to join with its neighbor of the opposite side in the formation of a band or chaplet in which the nasal bone will later be developed. It is quite evident that the frontal process is extending upward in this band, the inner surface of which is applied medially, near its upper extremity, against a portion of the ectethmoid which presents, upon the inner surface, the representative of the agger nasi. It is clear, therefore, that the frontal process later takes to itself the modeling of the cartilage here.

The outer surface of the process shows a few small foramina and at the apex the aforementioned unclosed end of the incisive suture appears. Its inner surface is largely occupied by the groove already described, together with its borders. There is a rough, spiculated area above and behind the groove. Just above the upper border of the process the nasolacrimal duct courses backward for some distance before turning downward to its termination. The lower border curves inward close to the paraseptal process of the ectethmoid, making of the post-transverse incisure a foramen.

The zygomatic process is represented by a thin plate of bone which ends in a dorsolateral spur (fig. 29) and which is separated from the nearest point of the zygomatic bone by a wide interval. It projects much more directly backward than in the adult. Its lateral edge is the direct extension of a ridge which forms the lateral side of the groove for the infraorbital nerve. Its anterior surface looks also somewhat upward. It narrows as it descends into the depths of the infraorbital groove. The posterior surface, as it passes downward, turns outward, widens, and goes over upon the lateral surface of the body. The medial border of the plate is continuous with the lateral edge of the tooth gutter (fig. 29). The process shows several irregular spicules of bone.

The groove for the infraorbital nerve is formed laterally by a forward extension of the lateral border of the zygomatic process and medially by a continuation backward of the upper border of the frontal process. It is narrower in front than behind and its floor is pierced by foramina carrying the superior alveolar nerves.

The alveolar process is as yet represented only by the crescentic, irregular, roughened edges of the tooth gutter (fig. 29). Beginning caudally but a little in front of the extremity of the zygomatic process, it sweeps forward and inward practically to the midline. The gutter is filled with developing teeth (fig. 30). The outer border, which separates the alveolar process from the facial surface, commences caudally as the medial margin of the zygomatic process. It is marked near this point by a small, backwardly projecting spur. Opposite this point there is a much larger spur of the same character upon the inner border. In front of this the two borders proceed to their craniomedial end with many slight but no great irregularities. The tooth gutter lies in almost a perfect plane. Its two borders are crossed near the middle, by an imperfect bridge (the interalveolar septum between the canine and the first milk molar) and in its depths are seen the superior alveolar nerves. The crescentic bar of tissue representing the developing teeth (figs. 30, 41) extends craniomedially almost as far as the midline and caudally a little beyond the bone. There are enlargements representing imperfectly the individual teeth.

The palatine process is rudimentary. It is seen best in figure 29 as a shelf of irregular bone fitted into the concavity formed by the inner border of the tooth gutter. Caudally, it comes close to but does not touch, the palate bone. The medial edge, of serrated appearance, is widely separated from its fellow of the opposite side and forms an obtuse angle with the caudal border. The lower surface, although irregular, contrives to form with the laterally lying alveolar process a surface which is, in general, plane (figs. 26, 29). The superior surface, on the contrary, slopes upward, especially in its cranial extremity, upon the body of the bone and is honeycombed by holes. It thus displays maxillary and premaxillary parts, the latter being very small. Upon the os incisivum there is, as yet, but slight trace of a palatine process.

Among other features of the mature bone which are represented in this maxilla may be briefly mentioned the nasal notch, which embraces the anterior end of the ectethmoid and appears as a sharp crescentic border running down from the lower border of the frontal process to the region of the future anterior nasal spine; also the infraorbital margin, passing backward and outward from the upper edge of the frontal process, crossing the infraorbital groove, and continuing upon the outer border of the zygomatic process.

The condensed mesenchyme enveloping the maxilla was modeled and is shown in figures 30 and 41. Its outlines are smooth and conform closely to those of the bone within. Its boundaries are somewhat indefinite in places. There are continuations with the mesenchyme of the maxilla of the opposite side in two places, viz, through an extension of the frontal processes over the bridge of the nose and through a connection between the anterior extremities of the alveolar processes of the two bones, which occurs just below the paraseptal cartilages. There is, too, a caudolateral connection from an extension of the zygomatic process to the mesenchyme of the zygomatic bone. The interior of the mass shows a lessening in density, where the fibers for the superior alveolar nerves traverse it. Seen from within in figure 41, we note that the anterior end of the alveolar process and (behind this) the palatine process are forming a floor for the inferior meatus, which, however, is complete only in front. Caudally, it will be observed, the mesenchyme of the maxilla comes into close contact with that of the palate bone, which, as yet, shows a very poorly developed palatine process.

A comparison of the maxilla of No. 886 with that of *Ia* shows at once that in the latter there have been noteworthy developments. Not only has the body become larger but the processes all show progress, the zygomatic reaching out to join that of the zygomatic bone, the alveolar projecting farther downward, and the palatine, with extension much farther toward the midline, being the most noteworthy. In the Hertwig model these growth features are still more evident, there being here almost a complete closure of the infraorbital foramen and an upward extension of the bone forming the medial boundary of the groove for the infraorbital nerve around the adjoining ectethmoidal cartilage.

PALATE.

The palate bone of No. 886 is represented by a thin plate of irregular outline bent into an inward concavity, situated between the maxilla and medial pterygoid plate (fig. 26). Although showing slight roughenings, the plate is remarkably smooth in comparison with the adjoining inner surface of the maxilla. In its medial concavity rests the mucous membrane of the lateral wall of the nose, as seen in figure 38. Though there is none of the elongation and little of the specialization of the adult condition, yet already many of the parts of the mature bone may be recognized. The main portion of the bone—a plate lying almost vertically—represents the pars perpendicularis. The inward concavity—seen in dorsoventral, but not in caudocranial planes—is found only in its caudal two-thirds and is due to the incurling of the upper and lower borders. The plate is pierced by two small foramina, carrying twigs from the palatine nerves, as shown in figure 26. It is continuous below with the horizontal part, behind with the pyramidal process, above with the sphenoidal and orbital processes, and in front with the maxillary process, all of which are as yet very rudimentary. The lateral surface (fig. 28), affording a less favorable view of these extensions, presents a shallow furrow, the pterygopalatine sulcus, deepening above to form the sphenopalatine notch. In it appear the orifices for the exit of the aforementioned two nerve-twigs. Though the middle of this surface shows an outward convexity in the coronal plane, corresponding to the concavity upon the medial surface, it also shows a marked outward concavity in the horizontal plane, due to the lateral projection of the caudal extremity—the representative of the pyramidal process.

The representative of the horizontal part, or palatine process, forms no angle with the perpendicular plate; on the contrary, there is simply a bending in toward the midline of the lower edge of the bone, especially marked in its caudal portion, shown by figure 29, giving a view of the bone from below. The cranial end of this part of the bone is separated from the palatine process of the maxilla by a distinct space (fig. 29).

The representative of the pyramidal process is the most lateral part as well as the most posterior part of the bone. It is separated from the horizontal part by an indentation and projects backward to the level of the most anterior edge of the medial pterygoid plate, but lies some distance lateral to this (fig. 29). It includes three spicules, of which the middle, terminating in a flattened point, is much the longer and is separated from those above and below by marked notches.

The upper border of the bone shows a sharp inbending opposite the nerve trunks descending from the sphenopalatine ganglion. This, the representative of the future sphenopalatine notch, marks the upper end of the pterygopalatine sulcus and separates the rudimentary orbital and sphenoidal parts. The former, overlaid by a small branch from the sphenopalatine ganglion, is seen in figure 27 from above and figure 26 from within. It is, as yet, only a roughened projecting edge, curving outward and forward from the sphenopalatine notch. The sphenoidal process, equally

rudimentary, rises upward and inward from this notch and is surmounted by the caudal end of the sphenopalatine ganglion. Its upper edge descends upon the uppermost spicule of the pyramidal process.

The maxillary process, representing the cranialmost part of the bone, is seen from within in figure 26, and from above in figure 27. It is flattened, its anterior edge is turned a little inward, and its ventral border projects downward toward the adjoining maxilla, giving to the process a hook-like appearance. Its upper portion fits into a notch upon the caudal aspect of the ectethmoid, between the caudal extremities of the maxillo-turbinate below and the planum antorbitale above, as figure 41 shows. There is a wide interval separating it from the maxilla. Seen from within (fig. 26), the caudal edge of the palatine process of the maxilla seems to fit into the notch which the lower border of the process makes with the perpendicular plate, but when seen from below (fig. 29) we note that there is a wide space separating the two bones. In *Ia* the bone was somewhat more advanced.

Views of the condensed mesenchyme enveloping the palate bone are seen in figures 30 and 41, and from these it will be noted that it is continuous with that of the medial pterygoid plate. This mesenchyme is shown, partially cut away, in figure 28. Its relation to the nasal cavity appears in figure 38. Its surfaces are somewhat more smooth than those of the bone, but the outlines of the two are everywhere in general agreement.

The *spheno-palatine ganglion* is, roughly, a three-sided pyramid of nerve cells with the base directed downward and the apex dorsocraniad. It lies in what will be the sphenopalatine fossa, with the sphenoidal process of the palate bone below, the processus alaris of the sphenoid dorsocaudal, and the ala temporalis lateral. A medial projection overlaps slightly the upper margin of the palate and thus encroaches somewhat upon the space from which it will later be excluded by the upward growth of the horizontal plate. Communicating with the caudal extremity of the base is the nerve of the pterygoid canal, while the medial side shows the two sphenopalatine branches from the maxillary nerve. The palatine nerves emerge from the base and lie in the pterygopalatine sulcus.

MEDIAL PTERYGOID PLATE.

The medial pterygoid plate has been described with the orbito-temporal region and its mesenchymal investment has also been referred to with the palate bone.

ZYGOMATIC.

The zygomatic bone is a rather thin plate of osseous tissue stretching between the zygomatic processes of the temporal and maxilla, but separated from both (figs. 3 and 5). Its lower edge is seen in figure 29. It is not divided into separate parts. It possesses four sharply defined angles, the dorsal, caudal, ventral, and cranial. Wide notches cut into the upper and lower borders of the plate, giving to it an S-shaped appearance. The bone lies with the lateral surface looking a little downward and forward, as well as outward. Many of the characters of the adult condition will be recognized. The dorsal angle, rounded and projecting upward, but separated from the frontal bone by a very wide space, represents the frontosphenoidal process. The temporal, the longest of the processes, which forms the caudal angle, is more slender and is directed upward, backward, and slightly outward. It does not overlap the zygomatic process of the temporal, as in *Ia*, but rather is separated by a short space from that process, as shown in figure 5. Thus the zygomatic arch is incomplete. Between the temporal and frontosphenoidal processes is the representative of the temporal border of the adult bone, a thin edge with a deep upward concavity. This curvature is more sharply marked than in the adult.

The malar tubercle forms the ventral angle and is the bluntest of all the processes. The edge of the bone joining it with the temporal process—representative of the masseteric border of the adult—pursues a more or less direct course, although marked by minor irregularities. The remaining angle, representing the infraorbital process, points cranially and a little medially and is separated from the malar tubercle by a well-marked incisure, directed downward, the representative of the portion of the bone which will later articulate with the maxilla. Between the infraorbital and frontosphenoidal processes the edge, representing the infraorbital border, is straight, except for slight roughenings. The lateral surface is fairly smooth and, in general, plane, with a slight outward concavity near the cranial end. The medial surface is similar and, in a position corresponding to the outward concavity, there is an inward convexity, which may be the representative of the orbital process. The thickest portion of the bone is in the region of the malar tubercle.

SQUAMA TEMPORALIS.

The squamous portion of the temporal bone consists of a thin narrow plate, placed just

lateral to the upper portions of the malleus and incus. It is prolonged in front into a slender point, and below into the long, spur-like zygomatic process. The squama proper is seen from the side in figure 5. Its lateral surface, quite smooth, shows a very slight convexity in the coronal plane and in the horizontal plane a more marked one, owing to the fact that the cranial end turns medially in such a marked manner. The rounded caudal end is only a short distance in front of the extremity of the short crus of the incus. The upper border, lying but a little below the upper border of the incus, pursues a fairly direct course forward to the level of the root of the zygomatic process, after which it curves rapidly downward. It is slightly serrated behind and markedly so in front (figs. 31, 34). The lower border, showing small irregularities, describes a gentle curve with concavity downward and passes over upon the zygomatic process. Just above the root of the zygomatic process the squama displays a small foramen, and from this point forward the plate becomes very narrow and thin and terminates in a slender process, with irregular saw-like edges, which projects downward, forward, and inward. It deviates widely from the zygomatic process, forming with this a deep notch. The medial surface of the squama, fairly smooth, and displaying concavities in the frontal and horizontal planes, is separated from the upper parts of the incus and malleus by but a narrow space.

The zygomatic process is very long and almost straight. It projects downward and forward, tapering gradually to a point which lies close to the tip of the temporal process of the zygomatic bone. This process is not so heavy as that of *Ia*, nor is the squama so wide. These differences are even more exaggerated in the Hertwig model.

GONIALE.

The goniale, seen in figures 31 and 34 as a separate bony element, has already been described in connection with the description of the malleus.

TYMPANIC BONE.

The tympanic bone (fig. 5) is so slightly developed as to be found only after careful search. It is a very small rounded nodule, consisting mainly of cells with but little ossified matrix. It appears from the side in the angle between the handle of the malleus and Meckel's cartilage, but is just outside the plane joining these structures. Its ventral tip is below and a little lateral to the goniale, while its posterior and larger end is below and extends a little medial to that bone, with which its mass of condensed mesenchyme is continuous. It is situated quite close to the lateral surface of the tympanic cavity and just in front of the developing external acoustic meatus. It is not nearly so far on in development as the tympanic of *Ia*, and even in the latter there was none of the ring-like form which is so characteristic of the new-born condition and which is well seen in the Hertwig model.

LACRIMAL.

The lacrimal bone is a very thin, narrow slip of osseous tissue which projects forward from the outer and upper edge of the posterior maxillary process (fig. 39), inclosed by a shell of mesenchyme. It measures scarcely 200 micra in length and is rather less than half as wide. The nasolacrimal duct lies just below it, as the figure shows. As we have noted in the description of the ethmoidal region, there is a long and club-shaped projection of cartilage, the paranasal cartilage, which points downward and forward from the ectethmoidal cartilage and thus forms a sharp notch between itself and the posterior maxillary process within, opening cranioventrally. The paranasal process occupies the position of the future hamulus lacrimalis, the lower salient end of the posterior lacrimal crest, and the notch within it would then become the sulcus lacrimalis. The lacrimal bone undoubtedly comes to occupy the position of the paranasal cartilage and the groove medial to it. As to the exact manner of its growth we do not have much information. It is usually described as arising from a single center, though Thompson (1907, Morris's Human Anatomy) states that not infrequently the hamulus is a separate element. In such a case it seems probable that this center would occur in or upon the paranasal process and would later fuse with the medial center around the lacrimal sulcus. Thompson also states (p. 75): "The hamular process is regarded as representing the remains of the facial part of the lacrimal seen in lower animals."

VOMER.

The vomer consists of two separate, very slender strips of bone which lie side by side along the lower border of the nasal cartilage, as shown in figure 10. They are widest in the middle portion (fig. 9), the ends tapering off to points. Only at the widest point do the bones reach the level of the lower edge of the nasal septum. The anterior ends curve a little upward. The thicker lower borders are closer together than

the upper, so that the two form a V-shaped trough which, however, is as yet open below. The upper edges are thin and suggest the edge of a knife. In *Ia* a narrow fusion between the lower borders has taken place near the anterior end, and it is well known that such a fusion soon occurs along the entire length of the bone, as the Hertwig model shows, the plates growing upward and hedging in the nasal septum. The two strips of bone are enveloped by a common sheath of mesenchyme, which is shown in part in figure 8. In this figure the anterior tips of the bones appear in their correct relation to the septum (which is shown in negative manner as a groove) and to the mesenchyme. The anterior tip comes into close relationship with the posterior end of the paraseptal cartilage, but lies closer to the mid-sagittal plane than this.

MANDIBLE.

The mandible is represented at this stage by paired plates of bone which lie just lateral to Meckel's cartilages. The right half alone has been modeled and is shown from without in figure 5. The extremities of the plate are wider than the intermediate portion, which shows somewhat below the middle a constriction. Here the lower end of the bone gives the appearance of having been bent forward upon the upper, forming a wide angle open cranially. The caudal border, in contrast to the cranial, shows scarcely any change in direction. Just below this constriction the large mental foramen appears and from this region the bone turns inward, its ventral extremity coming to lie very close to its partner of the opposite side, without, however, actually touching it. This inturning is represented in figure 3.

The upper end of the bone is shaped somewhat like a dagger, the point, which is notched, projecting toward the root of the zygomatic process of the squama temporalis—the region of the future mandibular fossa—from which, however, it is separated by a wide interval. This region, obviously, represents the future condyle. From it the boundaries rapidly diverge to projections of bone upon the caudal and cranial borders, which are almost opposite each other and which mark the extremities of the widest part of the mandible. The caudal projection, which also points medially, is the upper extremity of the inturned caudal edge or base of the mandible and represents the angle and the limit of the ramus here. The ventral delimitation of the ramus is not definite. The ventral projection, which shows a thin spur of bone directed upward and forward, represents the future coronoid process. Upon the border of the bone joining the coronoid and condyloid processes, there is a gentle upward concavity which is crossed, a little above, by the nerve to the masseter muscle. It represents the mandibular notch.

The lateral surface is fairly smooth, somewhat more so above than below. Due to the turning inward of the base, there appears on the lateral surface a rounded vertical ridge, which is best marked above and which, as it descends, flattens out and approaches the caudal border, which it meets about the level of the upper border of the mental foramen. Upon the medial surface there is a corresponding groove, seen in figures 33 and 35, as the upward continuation of the inferior alveolar groove. In it, as seen in figures 31 and 34, a portion of Meckel's cartilage lies, though the cartilage and bone are separated by a substantial interval, most marked above, where the groove is deepest; indeed, in the space between the condyle and Meckel's cartilage the auriculo-temporal nerve is found and it occupies only a small part of the available space. In front of the ridge upon the lateral surface there is a vertical groove, best marked above, whose cranial side passes forward upon the cranial border of the bone. A corresponding ridge occurs upon the inner surface. This bending of the plate gives to cross-sections of it an S-shaped appearance; indeed, such a shape is perceived by looking directly at the upper end of the bone.

The mental foramen is much larger relatively than that of the adult bone. It is roughly quadrilateral with the long axis dorsoventral. Its anterior, posterior, and upper boundaries are free and made of narrow, bony plates; its ventral border, on the contrary, is formed by the angle made by the junction of the medial alveolar wall with the lateral surface. It is a low ridge showing a sharp spike of bone at its cranial end. When the foramen is looked at directly from the side the medial alveolar wall obstructs the view completely. The plate of bone forming the dorso-cranial boundary of this foramen is thin and narrow. In front and behind, the lower part of the foramen opens into the V-shaped lower part of the inferior alveolar groove.

The surface of the bone immediately below the foramen is somewhat depressed, and there is a distinct groove passing downward from the lowest part of the opening to the ventral margin of the bone. This groove serves as the anterior delimitation of a small eminence of bone whose position justifies the conclusion that it represents

the mental tubercle. It is situated some distance below the mental foramen and forms a projection upon the lower border of the bone. Upon the corresponding inner surface there is a small depression. The lateral surface, in the region of the mental foramen and below it, is rather rough.

Turning now to a consideration of the medial wall, we note as its most prominent characteristic the plate of bone forming the medial wall of the inferior alveolar groove. This, the so-called splenial element, is highest in the region of the mental foramen. In front of this it becomes much thicker, and progressively lower, passing forward and inward to terminate as a thin spur of bone, with an extremity turned a little upward. Behind the foramen, too, the ridge decreases gradually in height and extends backwards rather more than two-thirds of the distance from the anterior tip of the bone to the angle, as shown in figures 33 and 35. The upper margin is much serrated and lies about on the same level as the lateral wall of the inferior alveolar groove, which also shows a very jagged edge. A little in front of the mental foramen there is a narrow bridge of bone joining the medial and lateral alveolar walls, which covers the incisive nerve, as shown in figure 34. The upper border of the medial alveolar wall, barring the irregularities mentioned, has a direction in general straight, with the exception of its caudal extremity, which turns upward a little. On the other hand, the attachment of the plate to the main portion of the mandible follows a curved line, with concavity upward and forward. This line crosses almost the entire bone, beginning in front not far from the anterior extremity of the mandible, and continuing backward with an increasingly strong inclination upward, almost to the posterior border of the bone. This accounts for the varying depth of the inferior alveolar sulcus—deep in the region of the mental foramen, and decreasing in front and behind—following the variation in height of the inner wall. Where the wall ends dorsocaudally the alveolar groove is continued as the groove formed by the inturning of the base of the bone, as far as the condyloid process (figs. 33 and 35).

Below the medial alveolar wall there is a region presenting a marked inward concavity. This is open behind, due to the fact that the lower border does not turn inward here, but rather projects outward to form a salient upon the lateral aspect. In this concavity rests the lower end of Meckel's cartilage (figs. 31 and 34). In the depths of this concavity there is an area, shown in figures 33 and 35, where the bone and Meckel's cartilage are in contact; indeed, the appearance is as though the perichondrium were becoming ossified here. The cartilage, too, in this region (fig. 32) shows evidence of calcification and is evidently undergoing the change preliminary to ossification. Similar findings were described by Low and were also reported in my former paper. The area marked involves the region of confluence of the medial alveolar wall with the main plate and extends a short distance downward from this.

In connection with the medial surface, the relation of Meckel's cartilage should be described. We have noted that there is a short portion of the rod which lies between the uppermost extremity of the mandible and the head of the malleus. Opposite the mandible it lies, as figures 31 to 35 show, in the groove which has been described. As it descends it comes to lie medial to the upper end of the medial alveolar wall and in close contact with this. It crosses the upper border of this wall at an angle, its cranial border falling somewhat behind and below the edge of the bone, as figures 31 and 34 show. From the angle of the mandible to its lower extremity the posterior edge is turned inward partially to envelop the cartilage, except in the region already described.

The position of the inferior alveolar nerve, as shown in figure 34, is interesting, as marking the position of the future canal. A short distance behind the coronoid process and below the mandibular notch, as shown in the figure, the nerve comes to lie quite close to the bone. At this point it lies just behind the ridge which has been mentioned and which occurs as a groove upon the outer surface. This region doubtless represents the site of the future mandibular foramen. The nerve, from this point, courses fairly close to the bone, crossing the ridge obliquely from behind forward, to divide, a little before the mental foramen is reached, into the mental and incisive branches. These nerves appear in figure 34, the former passing through the upper extremity of the mental foramen, the latter continuing downward, in the depths of the alveolar groove, to the tip of Meckel's cartilage.

The remainder of the mandibular nerve, in so far as it relates to the mandible, may be briefly described. The stout trunk seen in figures 31 and 34 shows, springing from almost the same region, the auriculo-temporal, masseter, lingual, and inferior alveolar branches. The auriculo-temporal, showing two strands, winds backward and outward, around Meckel's cartilage, and between it and the condyloid process, to emerge upon the face. The masseter has been referred to as passing over the representative

of the mandibular notch, some distance below and medial to the zygomatic process of the squama temporalis. The lingual is shown in the figures descending to the medial side of Meckel's cartilage, and being joined by the chorda tympani.

The borders of the mandible may be briefly summarized. Beginning above at the sharply marked condyloid process, and passing forward and downward, we traverse the mandibular notch—shallow when compared with that of the adult condition. From this the thin cranial edge of the bone is passed over. It is fairly smooth. Reaching the region of the mental foramen, the border becomes much more irregular, showing deviations from side to side and from above downward. There is a large notch just in front of the lower part of the foramen. This border terminates upon a sharp point projecting medially. Beginning again at the condyloid process, but proceeding backward, we pass downward over the dorsal border of the ramus. Between the condyloid process and the angle there is a strong outward convexity. From the angle downward the border becomes thicker and is somewhat roughened. As has been mentioned, it is turned inward, so as to present a salient edge when viewed from within (figs. 33 and 35). In *Ia* there was a very thin fissure visible upon the lateral surface which partially cut off this edge from the main plate. This fissure is only suggested in this specimen, as a very faint incutting of the bone, parallel to the caudal border and visible from without.

The turning inward of the base of the mandible does not occur below the level of the upper border of the mental foramen; on the contrary, when the bone is regarded from behind, it is seen that there is a sweeping outward of the border at and below this level, to form an outward projection. The curvature thus described marks the caudal limit of the concave area below the medial alveolar wall, already described. The border, continued downward and forward, passes over upon the symphysial portion, which shows fine serrations and gradually approaches its partner of the opposite side. This border ends, like the cranial, upon the ventrocranial tip of the bone.

The condensed mesenchyme of the mandible follows, in general, the outlines of the bone. It is confluent with its partner of the opposite side at the symphysis by a wide area, as shown in figure 36. The groove occupied by Meckel's cartilage is rather more conspicuous and, of course, there is a general increase in thickness of the mass.

The developing teeth, seen in figure 36, occupy the tooth gutter but do not extend as far back as the medial alveolar wall. As the figure shows, there are two main masses, connected by a narrow isthmus. This narrow point is just in front of the upper extremity of the mental foramen, at a point where we have noted the occurrence of a rod of bone joining the medial and lateral alveolar walls. The mesenchyme here is, of course, of greater volume than the inclosed bridge.

The *nasal* bone has not yet formed.

SUMMARY.

I. CHONDROCRANIUM.

General development.—The chondrocranium of No. 886 is well developed and is practically a homogeneous mass of cartilage, with but little trace of the regions of earlier separation of the parts. Almost all of it is represented.

Type of cartilage.—The cartilage is almost all of mature type. Young cartilage and precartilage are found principally at the anterior end, which is undergoing the most rapid development.

Evidences of preossification change.—In all, ten centers were found where the cartilage showed the change which ushers in ossification. Of these, four were paired and two unpaired. In none was actual bone formed. At the peripheries of these centers there was a gradual transition of the modified cartilage into the surrounding normal cartilage. The areas of these centers have been carefully indicated in the figures and have been described. They may be briefly recapitulated:

In the occipital region there are: (1) a single center for the basi-occipital; (2) paired centers for the exoccipitals; (3) a single large center for the supraoccipital.

In the orbito-temporal region there are: (1) paired centers in the temporal wings; (2) paired centers in the alar processes, these being very slightly developed.

The remaining paired centers are found one in each lower end of Meckel's cartilage.

Central stem.—The angle made by the chordal and prechordal parts of the central stem is 115°, as in *Ia*. It has become narrower since the 21 mm. stage of Lewis, where it was 125.

Orbital wings.—That the outer ends of the orbital wings become depressed, thus widening the angle made by the lines joining the limbus sphenoidalis with the lateral extremities of the wings, following the stage of No. 886, is evident from a comparison of this angle in No. 886 with the corresponding angle in the adult skull. This flattening of the floor of the anterior cranial fossa is probably associated with the growth of the brain.

Comparison of Chondrocranium of No. 886 with that of *Ia*.

The chondrocranium of No. 886 is considerably less developed than that of *Ia* and much of the cartilage is of a younger type. The points of contrast may be summarized as follows:

(*a*) *Parts absent from No. 886 and present in Ia.*
1. Supracochlear cartilages.
2. Superior paraseptal cartilages.
3. Lateral cranial cartilages.
4. Posterior cranial cartilages.

(*b*) *Parts present in No. 886 and absent from Ia.*
1. Alicochlear commissure. It was represented in *Ia* by a process. The carotid foramen was thus closed in No. 886 and open in *Ia*. From the alicochlear commissure is developed the lingula.

(c) *Parts showing a difference in No. 886 as compared with Ia.*

1. Styloid process. It is attached by cartilage to the otic capsule in No. 886, and is separate in *Ia*.
2. Mastoid process. It is a free nodule in No. 886 and was attached to the otic capsule by cartilage in *Ia*.
3. Paraethmoidal cartilage. It is attached to the ectethmoid by cartilage in No. 886 and is separated by membrane in *Ia*. It is very rudimentary on the right side.
4. Process of the middle meatus. This may represent the uncinate process. It is altogether precartilaginous in No. 886 and is long and slender. In *Ia* it is a nodule of very young cartilage with a pedicle of precartilage attaching it to the ectethmoid.
5. Paranasal process. It was a separate cartilaginous nodule in *Ia*.
6. Ala hypochiasmatica. Less developed in No. 886 than in *Ia*.
7. Prechiasmatic commissures. In No. 886 they are of precartilage and are very slender; in *Ia* they are cartilaginous and much stouter.
8. Prechiasmatic foramina. They are relatively larger in No. 886 than in *Ia*. They were found in the skull of a young human adult.

(d) *Parts found in No. 886 and not described in Ia.*

1. Cupular process. In No. 886 it is long, slender, and of precartilage. It partially encircles the epithelial plug in the anterior naris. Only a short projection of cartilage was described in *Ia*. This process develops into the medial crus of the greater alar cartilage (Peter, 1913). It is of interest that the process is so fully developed in precartilage at this stage.
2. Processus ascendens is represented by a spheroidal nodule attached to the upper border of the supraoccipital cartilage in No. 886. The corresponding region of *Ia* is missing.
3. Processus descendens is plainly marked in No. 886 and is not distinct in *Ia*.
4. Posterior petrosal process is represented in both skulls.
5. Anterior intraperilymphatic process. It is a very small projection.

II. ADDITIONAL CARTILAGES.

1. Parasphenoidal cartilage, or hamular process, is present and intimately associated with the medial, pterygoid plate.

2. Branchial-arch skeleton. The following cartilages have been described: Meckel's and Reichert's, with ossicle fundaments; hyoid; thyroid; arytenoid; cricoid; upper tracheal.

3. Cervical vertebræ. They show arch tips widely separated; those of the atlas are the same distance apart as those of the occipital vertebra.

4. Paraseptal cartilages and ventrolateral processes are rudimentary in comparison with those of *Ia*.

III. MEMBRANE BONES.

All of the membrane bones, with the exception of the nasal, are represented. Some of them are very small, as the medial pterygoid plate, the parietal, and particularly the interparietal, lacrimal, tympanic, and goniale. The palatine surface of the maxilla shows a groove indicating the line of earlier complete separation of the maxillary and premaxillary elements.

IV. NON-SKELETAL PARTS.

The following structures which are intimately related to the developing skull have been figured and described:

1. Notochord. It follows a typical course and perforates the anterior end of the preossification center for the basioccipital.

2. The outlines of the external form, brain, frontal, and parietal bones, and the chondrocranium, as seen from the right side, have been drawn by profile reconstruction, to show their common relationships.

3. Mucous membrane of the nose, showing relation to the nasal septum and vomeronasal organ and to the developing conchæ.

4. The vomeronasal organ is plainly indicated and is situated one upon either side of the septum. It is considerably above the paraseptal cartilages.

5. Nasolacrimal duct system. The lower end, not yet connected with the nasal cavity, is situated some distance behind the position which the corresponding structure occupies in the lower forms and its migration backward may be connected with the solution of continuity of the cartilaginous anterior transverse lamina.

6. The following ganglia have been mentioned, and some of them have been figured: semilunar, geniculate, sphenopalatine, cochlear, vestibular, jugular, and petrous of glossopharyngeal, jugular and nodosum of vagus, accessory.

7. The following nerves have been noticed and most of them have been figured: trigeminal and branches, facial, chorda tympani, great superficial petrosal, nerve of the pterygoid canal, glossopharyngeal, vagus, accessory, hypoglossal.

8. Auditory tube and tympanic cavity are shown in their relation to the otic capsule and ossicle primordia.

9. Internal carotid artery is shown in relation to the otic capsule, carotid foramen, and alar process of the developing sphenoid. Upon the alar process it lies in the representative of the carotid sulcus.

10. Membranous labyrinth and endolymphatic duct and sac. The slender extension from the last mentioned lies medial to the anterior end of the occipitoparietal groove.

11. Stapedius muscle and tendon are shown in relation to the styloid process, facial nerve, and surrounding structures.

In conclusion, I wish to express my thanks to Dr. G. L. Streeter, Director of the Carnegie Laboratory of Embryology, for the facilities of that Department, which were freely placed at my disposal.

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EXPLANATIONS OF FIGURES.

All drawings were made by Mr. James F. Didusch according to geometric projection. With the exception of figure 7, which was made from a profile reconstruction, all figures were drawn from the original plaster-of-paris models made from human fetus No. 886 of the collection of the Carnegie Laboratory of Embryology. The number of the model from which each figure was drawn is given, together with the magnification.

FIG. 1. Chondrocranium from above with frontal and parietal bones on right side. The densest part of the frontal bone is inclosed by a dotted line. The basal plate is not quite horizontal, the cranial end being a little the closer to the eye of the observer. Model 1. ×6.25.

FIG. 2. Chrondrocranium from below with cartilaginous branchial arch skeleton extirpated. Frontal and parietal bones are shown on right side. The basal plate is not quite horizontal, the caudal end being a little the closer to the eye of the observer. The view is directly into the anterior nares. Model 1. ×6.25.

FIG. 3. Skull from front, showing membrane bones on right side. Face is seen in frank view. The cervical vertebræ and cartilaginous branchial arch skeleton are also seen. Model 1. ×6.25.

FIG. 4. Skull from back, giving a frank view of the foramen occipitale magnum. The cervical vertebræ are seen, their arches being as yet unclosed dorsally. Note the alinement of the hemiarch tips with the dorsal foraminal prominences, representing the extremities of the hemiarches of the occipital vertebra. The right half of the interparietal bone is seen. Model 1. ×6.25.

FIG. 5. Skull from right side, showing membrane bones. The cervical vertebræ and cartilaginous branchial arch skeleton are included. Only the right half of the skull is shown. Model 1. ×6.25.

FIG. 6. Left half of chondrocranium, cervical vertebræ, and cartilaginous branchial arch skeleton as seen from left side. Model 1. ×6.25.

FIG. 7. Profiles of external form of head, brain and upper end of spinal cord, and skull, in their normal relation to one another, as seen from the right side. Drawn from a profile reconstruction. ×1.9.

FIG. 8. Condensed mesenchyme enveloping the vomer, seen from front, side, and above. The anterior extremities of the vomer are indicated. The gutter in the center is for the lower edge of the nasal septum. There is a slight amount of lateral curvature. The cut edges of the mesenchyme are indicated. Model 22. ×12.5.

FIG. 9. Two halves of the vomer from the same point of view as that of figure 8. They are very slender spicules of bone lying along the lower border of the nasal septum. Model 21. ×12.5.

FIG. 10. Median stem of skull as seen from right side. It consists of the basal plate behind and the interorbital and nasal septa in front, forming an obtuse angle at the body of the sphenoid. The adjoining exoccipital cartilage is shown in part. Junctions with cartilage lying laterally are shown. Model 4. ×12.5.

FIG. 11. Right half of basal plate and parts of upper two cervical vertebræ, sectioned in the midsagittal plane. The cut surface is seen in frank view. Shows the preossification center for the basioccipital, the notochord, the pharyngeal bursa with a little of the epithelium of the roof of the pharynx, the temporal wing, dorsum sellæ and a portion of the exoccipital. Model 8. ×12.5.

FIG. 12. Left cartilage of Jacobson from left side with neighboring septum. Models 2 and 25. ×12.5.

FIG. 13. Cartilages of Jacobson from below in relation to nasal septum. Models 2 and 25. ×12.5.

FIG. 14. Right half of occipital cartilage and parietal plate from in front and within, with the ascending process and right half of interparietal bone in its mesenchyme. Connections with adjoining cartilages are shown. Model 24. ×6.25.

FIG. 15. Interior of right otic capsule from within and above. The inner wall of the capsule has been cut away to show the lateral wall of the cavity. Figure 21 shows this cavity modeled as a solid. The superior and posterior semicircular canals are shown, with the entrances to the lateral canal. A good view is afforded of the spiral septum in the wall of the cochlear space. The membrane filling in the vestibular window is indicated. Other features are the medial end of the tuba auditiva with its entrance into the pharynx, the alar process of the temporal wing of the sphenoid, the alicochlear commissure, the internal carotid artery, and views of the fifth and seventh cranial nerves. Model 14. ×10.

FIG. 16. Right otic capsule and associated structures seen from right side, front, and a little below. The suprafacial commissure has been removed. The facial nerve is shown in its relation to the otic capsule and styloid process, with its off-shoots, the chorda tympani and the great superficial petrosal nerves, the latter arising from the geniculate ganglion. The tip of the long crus of the incus appears in relation to the chorda tympani. A prominent object is the tympanic cavity fundament, of which the lateral surface is shown, presenting an impression at the site of the future tympanic membrane. The auditory tube is shown in its full extent. The immense semilunar ganglion with its root and

branches, the internal carotid artery, and the processus alaris of the temporal wing of the sphenoid are seen. Model 14. ×10.

FIG. 17. Right otic capsule, medial surface, frank view, showing connections with adjoining cartilages and openings toward the cranial cavity. Model 5. ×10.

FIG. 18. The space within the right otic capsule seen from within, modeled as a solid; the surface presented fits into the cavity shown in figure 23. Openings toward the cranial cavity are shown. Note the large volume of this cavity in comparison with that of the membranous labyrinth which fills it (fig. 19). Compare also figures 21 and 22 in this respect. Model 6. ×10.

FIG. 19. Membranous labyrinth of right otic capsule contained within the space shown in figure 18. Figures 17, 18, and 19 were all drawn from approximately the same point of view, so that an accurate idea may be gained of the space contained within the otic capsule and the membranous labyrinth within that. Model 7. ×10.

FIG. 20. Right otic capsule, lateral surface, frank view, with openings looking outward. The attachments of the cartilage of Reichert and of the short process of the incus are seen. Model 5. ×10.

FIG. 21. The space within the right otic capsule, seen from without, modeled as a solid; the openings are indicated. Model 6. ×10.

FIG. 22. Membranous labyrinth of right otic capsule contained within the space shown in figure 21. Figures 20, 21, and 22 were all drawn from approximately the same point of view. Model 7. ×10.

FIG. 23. Medial wall of right otic capsule, seen from without, the lateral wall having been cut away. The cutting of the capsular wall was not done in quite the same way as in the model shown in figure 15, so that the cut edges do not fit together exactly. The inner wall of the space is seen with the endolymphatic and internal acoustic foramina. Model 26. ×10.

FIG. 24. View from front of mass of cartilage (massa angularis) partially inclosed by the semicircular canals of the right otic capsule. Above is seen the superior canal leading into the space for its ampulla and, farther downward and to the right, into that for the utriculus. To the left is the space for the ampulla of the lateral canal and, farther back, the lateral semicircular canal appears. Model 15. ×10.

FIG 25. View of same mass of cartilage shown in figure 24, but seen from below. The lateral semicircular canal is conspicuous to the left, passing above into an enlargement for the ampulla of this canal and for the utriculus, with the beginning of the superior canal above. Below, the lateral canal passes medially into an enlargement for the inferior extremity of the posterior canal and for the crus commune. Model 15. ×10. Views of the angular mass from other aspects are seen in other figures, as from without in figures 20, 5, 6, and from within in figure 15.

FIG. 26. Right maxilla, palate, medial pterygoid plate with hamular process, temporal wing, sphenopalatine ganglion, and associated nerves, seen directly from within. Cut surface showing junction of temporal wing with alar process is seen. Note the incisive suture partially separating the maxilla and premaxilla. Model 11. ×12.5.

FIG. 27. The same structures as those seen in figure 26 with the exception of the medial pterygoid plate, seen from above. The relations of the maxillary division of the trigeminal nerve and its branches to the different structures are shown. Model 11. ×12.5.

FIG. 28. Lateral aspect of the right palate bone and medial pterygoid plate, with their investment of condensed mesenchyme. Model 18. ×12.5.

FIG. 29. The same structures seen in figures 26 and 27, but viewed from below. The tooth gutter of the maxilla and premaxilla is conspicuous and the lower end of the incisive suture appears. The zygomatic bone also is shown. Compare with figure 30. Model 11. ×12.5.

FIG. 30. Condensed mesenchyme enveloping the right maxilla, palate, and medial pterygoid plate. The developing teeth are seen in their gutter in the maxilla. (Compare with figure 29 drawn from approximately the same point of view.) The cartilaginous hamular process is seen projecting from the medial pterygoid lamina. Compare also with figure 36, for developing teeth of the right lower jaw. Model 25. ×12.5.

FIG. 31. Frank view of the right mandible, Meckel's cartilage, and associated structures, seen from within. The cartilaginous precursors of the auditory ossicles are seen above, in relation to the facial and chorda tympani nerves. A glimpse of the squama temporalis is given and also of the goniale. Note the relations of the mandibular division of the trigeminal nerve. Model 9. ×12.5.

FIG. 32. Lateral aspect of lower end of the right Meckel's cartilage, showing especially the area applied closely to the mandible, where the cartilage is showing the changes preliminary to ossification. (Compare with figures 33 and 35.) Model 16. ×12.5.

FIG. 33. Right mandible from same viewpoint as in figure 31, showing the tooth gutter and area of close apposition to the lower end of Meckel's cartilage. Model 10. ×12.5.

FIG. 34. The same structures as those shown in figure 31, but the model was rotated somewhat medially around its long axis. While presenting all the structures from a new angle, it shows especially the relation of the nerves to the mandible. Model 9. ×12.5.

FIG. 35. View of right mandible with the model in the same position as that shown in figure 34; it is rotated so as to afford a good view of the tooth gutter. Model 10. ×12.5.

FIG. 36. Condensed mesenchyme around right mandible viewed from approximately the same point as that of figure 35. It shows the developing teeth of the right lower jaw. The mesenchyme is connected across the midline with its partner of the opposite side, the cut edge being shown. Model 23. ×12.5.

FIG. 37. Mucous membrane of inner wall of right nasal cavity, overlying the septum; it is cut away to show the right organ of Jacobson. (Compare with figure 10.) Model 20. ×12.5.

FIG. 38. Mucous membrane of lateral wall of right nasal cavity, overlying the right ectethmoid, showing folds for the developing conchæ. The mucous membrane fits over the structures seen in figure 41. The elongated nasopharyngeal canal, flanked by the developing palate and medial pterygoid plate, is well seen. Model 18. ×12.5.

FIG. 39. Lateral aspect of right ectethmoid from the front, side, and a little below, showing especially the nasolacrimal duct, with the nasolacrimal sac and the lacrimal ducts above, and, below, the expanded end, applied to but not perforating the external aspect of the mucous membrane of the inferior meatus. The tip of the paranasal cartilage lies just lateral to the duct and the tiny shred of osseous tissue representing the lacrimal bone is seen lying along the posterior maxillary process. The cupular process of precartilage is conspicuous in the lower part of the figure. The broad plate of epithelium, which represents the future inferior meatus, but which has not yet undergone cleavage except posteriorly, is plainly shown. Model 18. ×12.5.

FIG. 40. Anterior end of right ectethmoid, with the epithelial plug in the anterior naris, embraced medially by the cupular process of precartilage. The terminal portion of the nasolacrimal duct is shown, entering the space for the inferior meatus, with a small portion of the mesenchyme of the maxilla. Model 19. ×12.5.

FIG. 41. Medial aspect of right ectethmoid, showing the developing conchæ. Precartilage is especially evident in the superior concha, the small process of the middle meatus, and the cupular process. The other conchæ are edged with it. The mesenchyme envelopes of the maxilla, the palate, and the medial pterygoid plate are seen. The cartilaginous hamular process is conspicuous, as are also the developing teeth of the right side of the upper jaw. (Compare figure 30.) Note also the tip of the nasolacrimal duct in the space for the inferior meatus. Model 25. ×12.5.

FIG. 42. View of right hyoid arch from without, below, and behind. The connection with the otic capsule is seen above and below appears the lesser cornu of the hyoid cartilage. The relations of the facial nerve, chorda tympani, and tympanic cavity are well seen, and the handle of the malleus is plainly shown in a concavity representing the stratum mucosum of the future tympanic membrane. Model 17. ×12.5.

FIG. 43 Medial aspect of left ectethmoid (compare with figure 41), showing developing conchæ. The anterior portion of the tectum nasi has been trimmed a little farther laterally than on the right side, and hence the cut surface is not quite the same in the two figures. The cupular process is omitted. Model 3. ×12.5.

FIG. 44. View of right hyoid arch from within and slightly above, with its membranous connection with the lesser cornu of the hyoid below (as in fig. 42) and the cut edge of its connection with otic capsule above. Fitting into the curvature of its upper portion is the epithelium of the developing tympanic cavity, which from this point of view is almost parallel with the plane of the paper. The ring-like stapes is seen and to it is attached the tendon of the stapedius muscle, with the muscle itself passing medial to the facial nerve and to the upper end of the styloid process. The handle of the malleus is also seen, with the chorda tympani lying just medial to it. Model 17. ×12.5.

FIG. 45. Cartilages of the hyoid, thyroid, cricoid, and upper end of the trachea, seen from the front. On the left side of the model the precartilaginous edging of the upper three cartilages is shown. Model 12. ×12.5.

FIG. 46. Right half of the same structures seen in figures 45 and 47, viewed from within. The precartilage of the arytenoid is seen and should be compared with the young cartilage of the same structure shown in figure 47. Model 13. ×12.5.

FIG. 47. Same structures seen in figure 45, but viewed from behind. The asymmetry evident in the arytenoids and in other places is due to the fact that on the left side of the model the precartilage was shown, whereas on the right side only the cartilage and young cartilage appear. Model 12. ×12.5.

ABBREVIATIONS.

ala hypoch.	ala hypochiasmatica
ala orb.	ala orbitalis
ala tem.	ala temporalis
am. can. lat.	ampulla canalis semicircularis lateralis
am. can. post.	ampulla canalis semicircularis posterior
am. can. sup.	ampulla canalis semicircularis superior
am. mem. lat.	ampulla membranacea lateralis
am. mem. post.	ampulla membranacea posterior
am. mem. sup.	ampulla membranacea superior
ang.	angulus
ar.	area in close apposition to the beginning ossification center in Meckel's cartilage
arc. neur. occ.	arcus neuralis vertebræ occipitalis
art. car. int.	arteria carotis interna
bur. phar.	bursa pharyngea
can. coc.	canalis spiralis cochleæ
can. lat.	canalis semicircularis lateralis
can. n. hypo.	canalis nervi hypoglossi
can. post.	canalis semicircularis posterior
can. sing.	canalis singularis
can. sup.	canalis semicircularis superior
caps. ot.	capsula otica
cart. ary.	cartilago arytænoidea
cart. basiocc.	cartilago basioccipitalis
cart. basisphen.	cartilago basisphenoidalis
cart. cric.	cartilago cricoidea
cart. hy.	cartilago hyoidea
cart. Meck.	cartilago Meckeli
cart. parasep.	cartilago paraseptalis
cart. parasep. lat.	cartilago paraseptalis lateralis
cart. parasep. med.	cartilago paraseptalis medialis
cart. parasph.	cartilago parasphenoidalis
cart. sph. eth.	cartilago sphenoethmoidalis
cart. supraocc.	cartilago supraoccipitalis
cart. thy.	cartilago thyreoidea
cart. trach.	cartilagines tracheales
cav. tym.	cavum tympani
ch. dor.	chorda dorsalis
ch. ty.	chorda tympani
com. alicoc.	commissura alicochlearis
com. basicoc.	commissura basicochlearis
com. caps. occ.	commissura capsulo-occipitalis
com. præch.	commissura præchiasmatica
com. sph. eth.	commissura sphenoethmoidalis
com. sph. par.	commissura sphenoparietalis
com. suprafac.	commissura suprafacialis
conc. inf.	concha nasalis inferior
conc. med.	concha nasalis media
conc. sup.	concha nasalis superior
cond. occ.	condylus occipitalis
cor. maj.	cornu majus
cor. min.	cornu minus
cris. arc. occ.	crista arcuata occipitalis
cris. gal.	crista galli
cris. par.	crista parotica
cris. trans.	crista transversa
crus com.	crus commune
crus long.	crus longum
cu. al. hy.	cut edge of ala hypochiasmatica
cu. caps. ot.	cut edge of connection with otic capsule
cu. cart. parasep.	cut edge of connection with paraseptal cartilage
cu. com. caps. occ.	cut edge of capsulooccipital commissure
cu. com. caps. par.	cut edge of capsuloparietal commissure
cu. com. præch.	cut edge of prechiasmatic commissure
cu. com. suprafac.	cut edge of suprafacial commissure
cu. ect. eth.	cut edge of connection with ectethmoid
cu. inc.	cut edge of connection with incus
cu. proc. alar.	cut edge of connection with alar process
cu. proc. cup. ant.	cut edge of root of anterior cupular process
cu. proc. sty.	cut edge of connection with processus styloideus
cu. rad. metop.	cut edge of radix metopticus of ala orbitalis
cu. rad. præop.	cut edge of radix præopticus of ala orbitalis
cu. sep. nasi.	cut edge of connection with nasal septum
cu. tec. post.	cut edge of tectum posterius
den.	dentes
dor. scl.	dorsum sellæ
duct. coc.	ductus cochlearis
duct. endolym.	ductus endolymphaticus
duct. lac.	ductus lacrimale
duct. lat.	ductus semicircularis lateralis
duct. nas. lac.	ductus nasolacrimalis
duct. post.	ductus semicircularis posterior
duct. reun.	ductus reuniens
duct. sup.	ductus semicircularis superior
em. occ. lat.	eminentia occipitalis lateralis
epis.	epistropheus
epith. pl.	epithelial plug in anterior naris
fen. crib.	fenestra cribrosa
fen. perilym.	fenestra perilymphatica
fen. vest.	fenestra vestibuli
fis. orb. nas.	fissura orbitonasalis
fis. sph. par.	fissura sphenoparietalis
for. caps. occ.	foramen capsulooccipitale
for. caps. par.	foramen capsuloparietale
for. car.	foramen caroticum
for. coc.	foramen cochleare
for. endolym.	foramen endolymphaticum
for. epiph.	foramen epiphaniale
for. fac.	foramen faciale
for. jug.	foramen jugulare
for. men.	foramen mentale
for. occ. mag.	foramen occipitale magnum
for. optic.	foramen opticum
for. paracond.	foramen paracondyloideum
for. præch.	foramen præchiasmaticum
for. rot.	foramen rotundum
for. vest. inf.	foramen vestibulare inferior
for. vest. sup.	foramen vestibulare superior
fos. cond.	fossa condyloidea
fos. hypoph.	fossa hypophyseos
fos. mal.	fossa manubrii mallei
fos. subar.	fossa subarcuata
fron.	os frontale
gang. genic.	ganglion geniculi
gang. semilun.	ganglion semilunare
gang. sph. pal.	ganglion sphenopalatinum
gon.	goniale
ham. ptery.	hamulus pterygoideus
imp. sep. spir.	impression for the spiral septum
inc.	incus
inc. cond. post.	incisura condyloidea posterior
inc. intercond.	incisura intercondyloidea
inc. occ. par.	incisura occipitoparietalis
inc. occ. sup.	incisura occipitalis superior
incis.	os incisivum
interpar.	os interparietale
lac.	os lacrimale
lam. med. pteryg.	lamina medialis processus pterygoidei
lam. par.	lamina parietalis

m. stap.	musculus stapedius
mal.	malleus
man.	mandibula
manub.	manubrium mallei
mas. ang.	massa angularia
max.	maxilla
meat. ac. int.	meatus acousticus internus
mem. muc.	membrana mucosa
mes. incis.	condensed mesenchyme of incisive bone
mes. interpar.	condensed mesenchyme of interparictal bone
mes. man.	condensed mesenchyme of mandible
mes. max.	condensed mesenchyme of maxilla
mes. pal.	condensed mesenchyme of palate bone
mes. vom.	condensed mesenchyme of vomer
n. alv. inf.	nervus alveolaris inferior
n. aur. tem.	nervus auriculotemporalis
n. fac.	nervus facialis
n. infraorb.	nervus infraorbitalis
n. ling.	nervus lingualis
n. man.	nervus mandibularia
n. max.	nervus maxillaris
n. men.	nervus mentalis
n. ophth.	nervus ophthalmicus
n. pet. superfic. maj.	nervus petrosus superficialis major
o.	orificium, in the otic capsule, the result of incomplete chondrification
orb.	orbita
org. vom. nas.	organon vomeronasale
os. basiocc.	preossification center of the basioccipital cartilage
os. exocc.	preossification center of the exoccipital cartilage
os. Meck.	preossification center of Meckel's cartilage
os. supraocc.	preossification center for the supraoccipital cartilage
pal.	os palatinum
par.	os parietale
præcart. ary.	præcartilago arytænoidea
proc. alar.	processus alaris
proc. alar. sup.	processus alaris superior
proc. art. sup.	processus articularis atlantis superior
proc. asc.	processus ascendens
proc. cond.	processus condyloideus
proc. coron.	processus coronoideus
proc. cup. ant.	processus cupularis anterior
proc. cup. post.	processus cupularis posterior
proc. desc.	processus descendens
proc. intraper. ant.	processus intraperilymphaticus anterior
proc. intraper. post.	processus intraperilymphaticus posterior
proc. mast.	processus mastoideus
proc. max. post.	processus maxillaris posterior
proc. meat. med.	processus meatus medii
proc. paracond.	processus paracondyloideus
proc. paranas.	processus paranasalis
proc. parasep.	processus paraseptalis
proc. sty.	processus styloideus
proc. ven. lat.	processus ventrolateralis
proc. zyg.	processus zygomaticus
prom.	promontorium
prom. am. post.	prominentia ampullaris posterior
prom. dor. for.	prominentia dorsalis foraminis magni
prom. nas. sup.	prominentia nasi superior
rad. n. trig.	radix nervi trigemini
ram. incis.	ramus incisoris
rec. utric.	recessus utriculi
sac.	sacculus
sac. endolym.	saccus endolymphaticus
sac. lac.	saccus lacrimalis
sep. interorb.	septum interorbitale
sep. nasi	septum nasi
sep. spir.	septum spirale
sp. ut.	spatium utriculi
sq. tem.	squama temporalis
st.	stapes
sul. occ. par.	sulcus occipitoparictalis
sul. sep.	sulcus septalis
sul. suprasep.	sulcus supraseptalis
sut. inc.	sutura incisiva
teg. tym.	tegmen tympani
tub. aud.	tuba auditiva (Eustachii)
tub. jug.	tuberculum jugulare
tub. sel.	tuberculum sellæ
tun. muc. phar.	tunica mucosa pharyngea
tym.	os tympanicum
vom.	vomer
zyg.	os zygomaticum
3, 4, 5, 6, 7,	vertebræ cervicales

COLOR SCHEME OF PLATES.

In general, blue is used to indicate cartilage and precartilage, yellow to indicate bone, and green for beginning ossification centers. Cut edges are white. The chief departures from this scheme are as follows:

Plate 2.—Figure 7, brain green; figure 8, mesenchyme green; figure 11, mucous membrane of pharynx yellow; figures 12 and 13, paraseptal cartilage and precartilage green.

Plate 3.—Figures 15 and 16, mucous membrane of auditory tube yellow, nerves and ganglia green. Figures 17, 20, and 23, young cartilage green; figures 18 and 21, cavity of otic capsule green; figures 19 and 22, membranous labyrinth yellow.

Plate 4.—Figures 28, mesenchyme green; figures 30 and 36, developing teeth yellow, mesenchyme green; figures 33 and 35, ossifying perichondrium green.

Plate 5.—Figures 37, 38, 40, 42, and 44, mucous membrane yellow, precartilage green; in figure 37, epithelium of Jacobson's organ green; in figures 38 and 40, mesenchyme white; in figure 41, teeth yellow, precartilage green, mesenchyme and epithelium of nasolacrimal duct white. Figures 43, 45, 46, and 47, precartilage green.

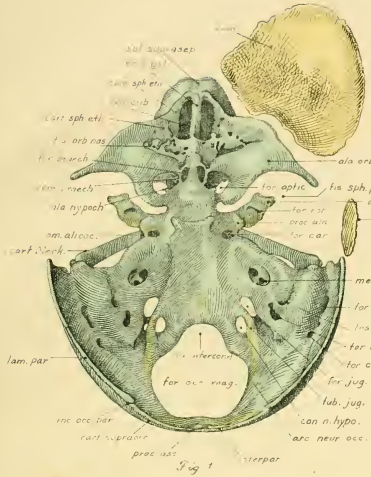


Fig 1

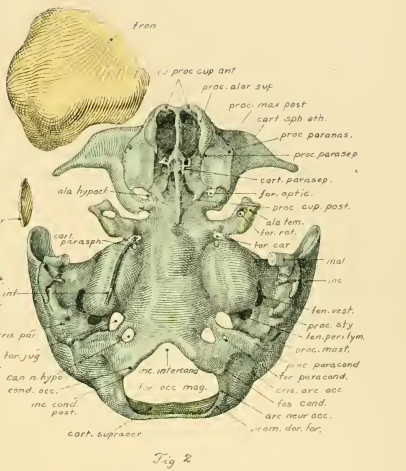


Fig 2

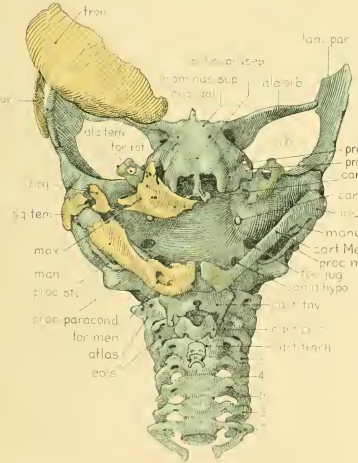


Fig 3

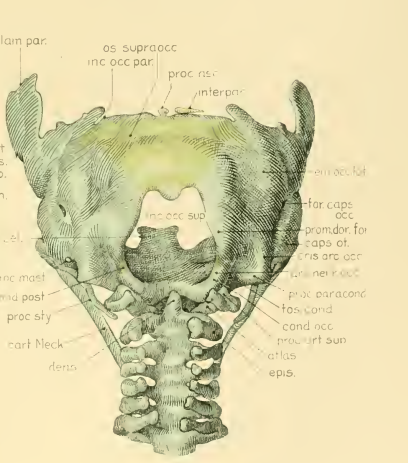
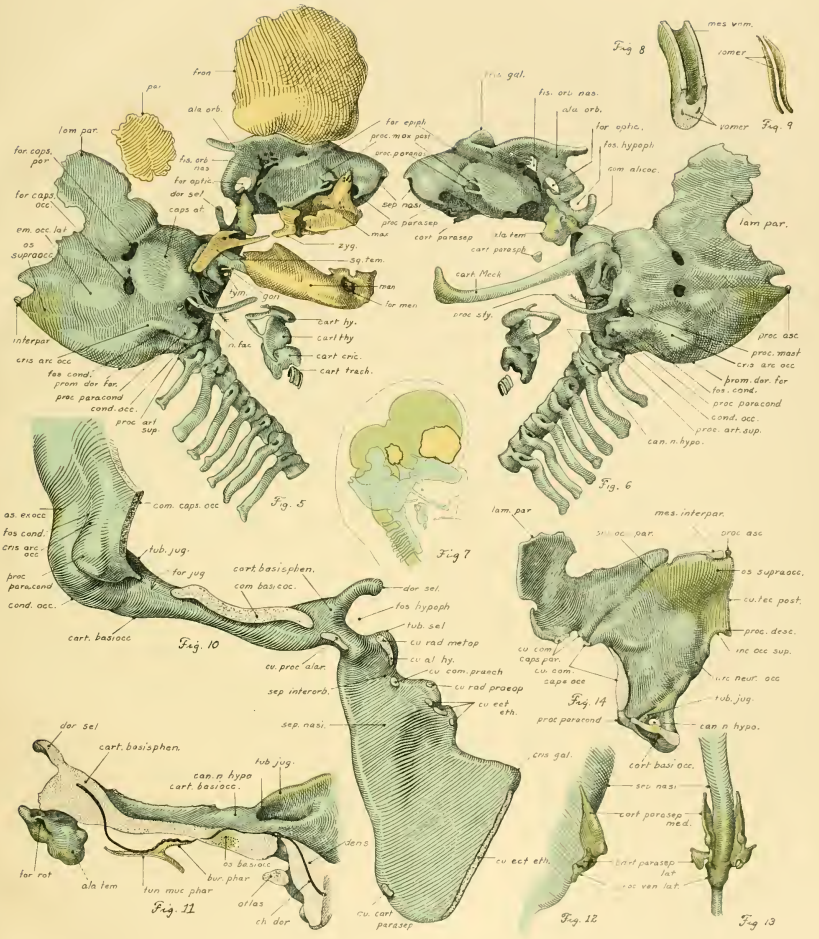


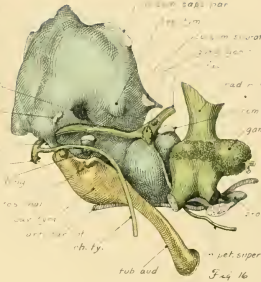
Fig 4

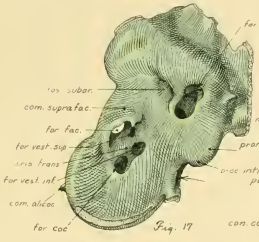


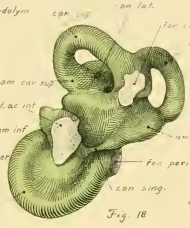
J. F. Didusch fec.

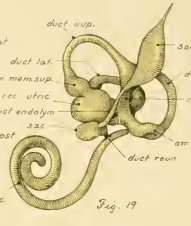
A. Hoen & Co. Lith.

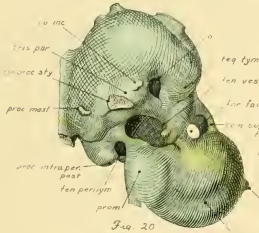

fos subar
com supra fac
for fac
gang semilun
n ophth
n max
mast
art cur int
com alicoc
proc alar
tub aud
fen vest
proc intraper post
Fig. 15

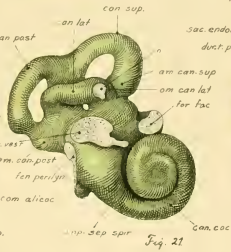

com supra fac.
gang. semilun.
proc
n fac
tub aud
Fig. 16

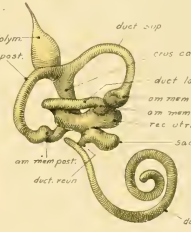

for endolym
fos subar.
com. supra fac.
for fac.
for vest sup
for vest inf.
com alicoc
for coc
Fig. 17

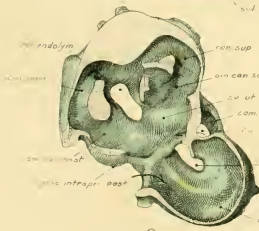

for endolym
can post
am can sup
meat ac int
prom am inf
proc intraper post.
fen perilym
con sing.
con coc
Fig. 18


duct sup.
sac endolym.
duct lat.
duct post
am mem sup.
rec utric.
crus com
duct endolym
sac
am mem post.
duct reun
duct coc.
Fig. 19

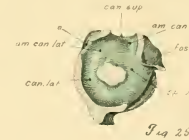

fen vest.
for fac
proc mast
proc intraper post
fen perilym
prom
sup sep
Fig. 20

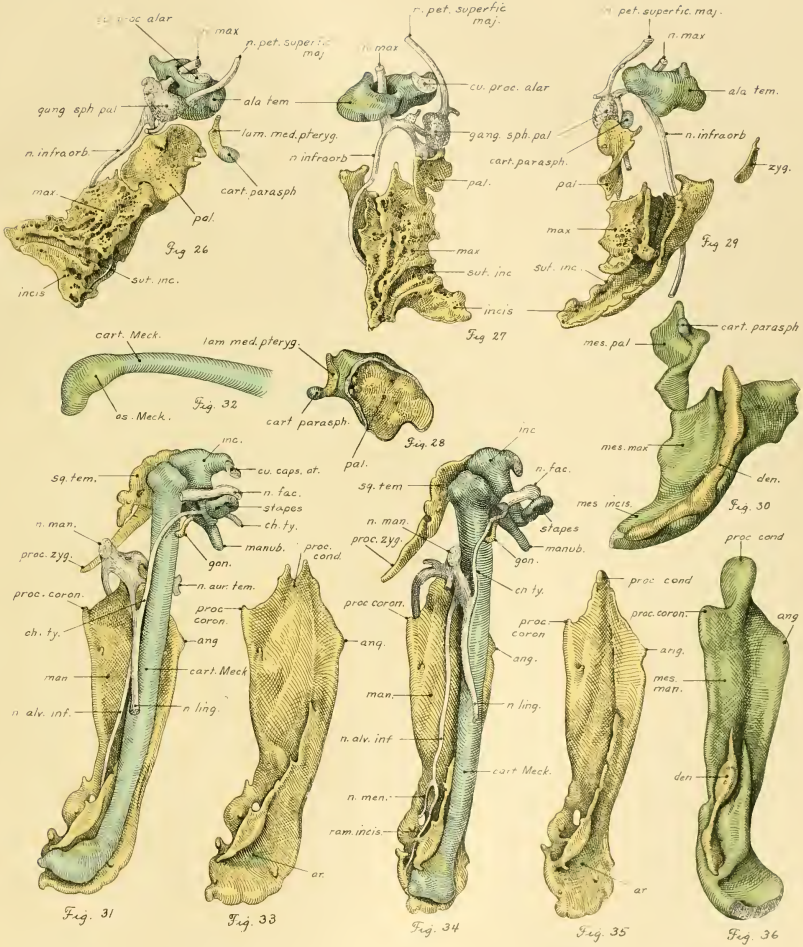

con sup.
an lat
can post
am can sup
om can lat
for fac
fen vest
am can post
fen perilym
com alicoc
sup sep spir
com coc
Fig. 21


sac endolym
duct post
duct sup
crus com.
duct lat.
am mem sup.
am mem lat.
rec utric.
sac
am mem post.
duct reun
duct coc
Fig. 22


con sup
am can sup.
com supra fac
for fac
meat ac int
com alicoc
com coc
Fig. 23


can sup.
fos subar
sp ut
can lat
am can lat
Fig. 24


can sup
am can sup
am can lat
fos subar
can lat
Fig. 25


proc alar
max
n. pet. superfic maj
gang sph pal
ala tem
lam. med. pteryg.
n. infraorb
max
cart. parasph
pal.
sut. inc.
incis
Fig 26
n. pet. superfic maj.
n. max
cv. proc. alar
gang. sph. pal
n. infraorb
cart. parasph.
pal.
max
sut. inc
incis
Fig 27
n. pet. superfic. maj.
n. max
ala tem.
n. infraorb
zyg.
pal
max
sut. inc.
Fig 29
cart. Meck.
os. Meck.
Fig. 32
lam. med. pteryg.
cart. parasph.
pal.
Fig. 28
cart. parasph
mes. pal
mes. max
den.
mes. incis.
Fig. 30
inc.
sq. tem.
cv. caps. at.
n. fac.
stapes
ch. ty.
n. man.
manub.
proc. zyg.
gon.
n. aur. tem.
proc. coron.
ch. ty.
ang.
man
cart. Meck
n. alv. inf.
n. ling.
proc. cond.
proc coron.
ang.
ar.
Fig. 31
Fig. 33
inc
sq. tem
n. fac.
n. man
stapes
manub.
proc. zyg.
gon.
ch ty.
proc coron.
proc coron
ang.
man
n ling.
n. alv. inf
cart Meck.
n. men.
ram. incis
Fig. 34
proc cond
proc. coron.
ang.
mes. marg.
den
ar
Fig. 35
proc cond
ang
den
Fig. 36

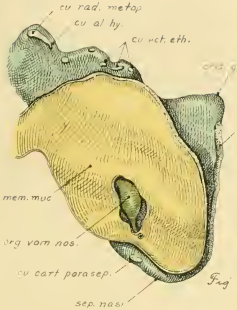


Fig. 37

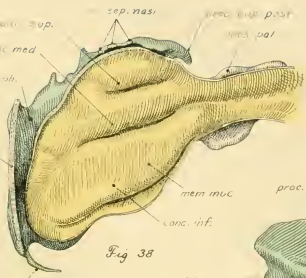


Fig. 38

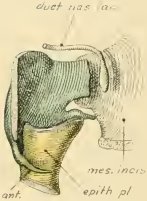


Fig. 40

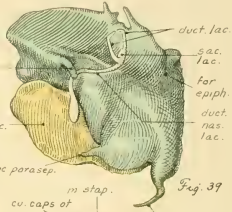


Fig. 39

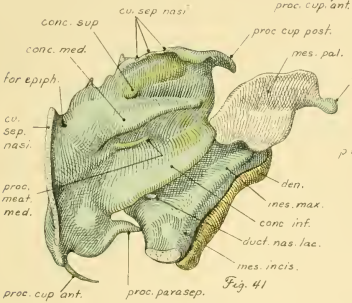


Fig. 41

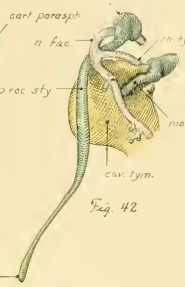


Fig. 42

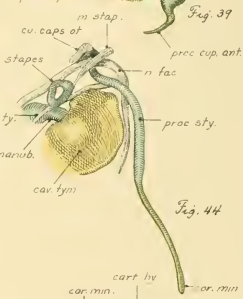


Fig. 44

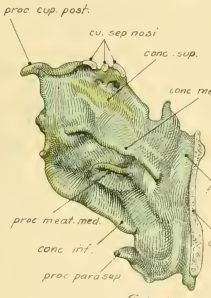


Fig. 43

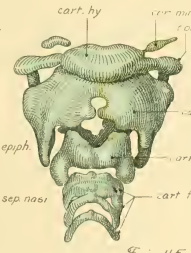


Fig. 45

Fig. 46

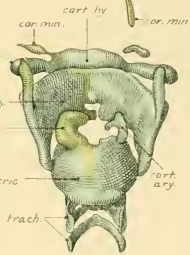


Fig. 47

CONTRIBUTIONS TO EMBRYOLOGY

VOLUME X, NOS. 47 AND 48.

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